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Formulation, and physical, *in vitro* and *ex vivo* evaluation of transdermal ibuprofen hydrogels containing turpentine oil as penetration enhancer

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The aim of the present study was to investigate the transdermal permeation enhancing capability of turpentine oil for ibuprofen from hydrogels. Ibuprofen 1% w/v hydrogels were developed with carboxypolymethylene with and without turpentine oil. Turpentine oil was incorporated in increasing concentrations, i.e. 0.5, 1, 1.5, 2, 2.5 and 3% of the total gel formulation, and its permeation enhancing effect was examined. Gels were examined physically for pH, viscosity, spreadability, extrudability, smoothness and appearance. To study the *in vitro* and *ex vivo* permeation potential of formulated gels, permeation studies were performed with a Franz diffusion cell using cellulose membrane and excised rabbit abdominal skin. Ibuprofen hydrogel with 3% turpentine oil showed a maximum flux of 10.87 mg/cm^2/h across artificial skin and 17.26 mg/cm^2/h across rabbit abdominal skin.

1. Introduction

Ibuprofen is a well established non-steroidal anti-inflammatory drug which is used for the symptomatic treatment of rheumatoid arthritis, ankylosing spondylitis, dysmenorrhea, etc. Given that ibuprofen is often used long-term, transdermal delivery might be more appropriate to reduce the side effects while maintaining its therapeutic blood concentration. However, it is difficult to maintain effective blood concentrations by transdermal delivery of ibuprofen due to its intrinsically poor skin permeability (Yano et al. 1986). Therefore evaluation of the potential for enhancement of the skin permeation of ibuprofen is of great practical importance.

Transdermal drug delivery systems present a number of advantages over traditional drug delivery methods. All these advantages have been quoted by various authors and include avoidance of first pass metabolism, decrease in side effects (e.g. gastric irritation), improved patient compliance, and enhanced therapeutic efficacy (Stott et al. 1998).

Chemical penetration enhancers modify the barrier properties of the stratum corneum and hence increase drug permeability across the skin. Ideally, the effects of a penetration enhancer on the skin should be reversible, and the enhancer should be non-toxic, nonallergenic, compatible with drugs and excipients and nonirritating. Many enhancers have these properties e.g. azone and its analogues (Michniak et al. 1993), fatty acids (Aungst et al. 1990), alcohols (Takahashi et al. 1991), and pyrrolidones (Babu et al. 2008).

Due to their systemic and localised toxicity, many effective permeation enhancers have not yet been employed (Palma et al. 2006). Hence, there has been increasing use of natural products as enhancers, due to their better safety profile.

Terpenes are essential oils, which are used as fragrances, flavourings, and medicines. They have been found to be effective penetration enhancers for a number of hydrophilic and lipophilic drugs (Charoo et al. 2008).

Table 1: Gel formulations of IBF

F. Code	IBF	CPM	TEA	Ethanol	T. Oil	D. Water
P1	1 g	1 g	1 ml	10 ml	–	QS to 100 ml
P2	1 g	1 g	1 ml	10 ml	0.5 ml	QS to 100 ml
P3	1 g	1 g	1 ml	10 ml	1 ml	QS to 100 ml
P4	1 g	1 g	1 ml	10 ml	1.5 ml	QS to 100 ml
P5	1 g	1 g	1 ml	10 ml	2 ml	QS to 100 ml
P6	1 g	1 g	1 ml	10 ml	2.5 ml	QS to 100 ml
P7	1 g	1 g	1 ml	10 ml	3 ml	QS to 100 ml

2. Investigations, results and discussion

2.1. Solubility study

At this point, an attempt was made at this point to discover which solvent is best able to maintain sink conditions in both dissolution and permeation studies. The highest concentration determined was 0.059 mg/ml in pH 7.2 phosphate buffer. Thus, pH 7.2 phosphate buffer was chosen as the dissolution and permeation medium because a sufficient amount of drug dissolved in it as is necessary to maintain sink conditions. The solubility of IBF in different solvents is given in Table 2.

2.2. Physical evaluation of gels

As shown in Table 3, the pH values of all the hydrogels developed ranged from 6.2 to 6.9, which lies in the normal pH range

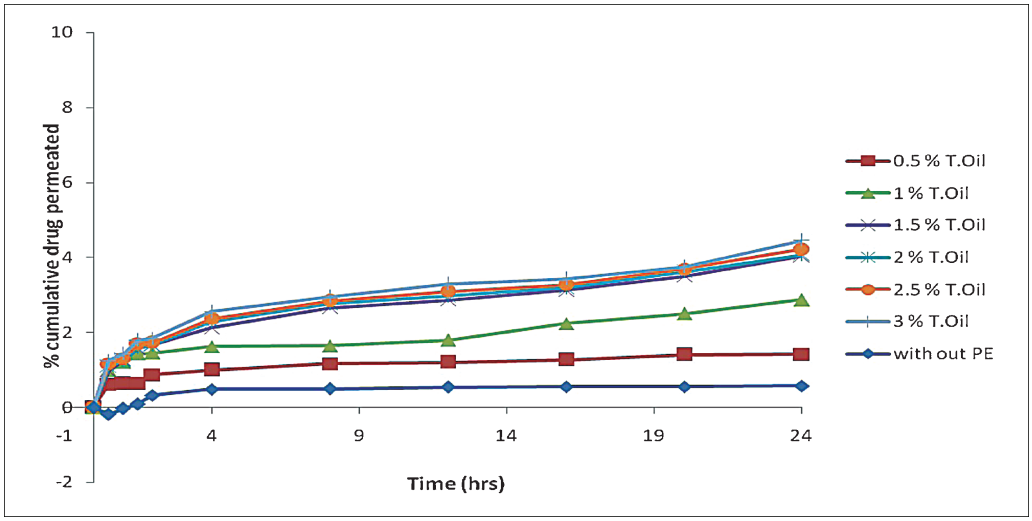

Fig. 1: Cumulative permeation of IBF gel across rabbit skin.

Table 2: Solubility study of IBF at 37 °C

Solvent	Mean of 3 absorbance values	Concentration (mg/ml) ± SD
Phosphate buffer pH 6.8	2.635	0.057 ± 0.02
Phosphate buffer pH 7.2	2.635	0.059 ± 0.04
Phosphate buffer pH 7.4	2.762	0.058 ± 0.03

and would not produce any skin irritation. The drug content of all the formulations ranged from 88.9 to 95.7%, which shows content uniformity. All the hydrogels developed showed good homogeneity with an absence of lumps. All the preparations were clear. The skin irritation studies of the hydrogels carried out on human volunteers confirmed the absence of any erythema or edema on the surface to which they were applied during 48 hr after application, as shown in Table 3. Spreadability results for the different hydrogels varied from 5.57 to 6.80 g.cm/sec (Table 4). The spreadability values indicate that the hydrogel is easily spreadable by a small amount of shear. Extrudability reflects the capacity of the hydrogel to be ejected in uniform and desired quantity when the tube is squeezed. Extrudability in terms of weight in grams varied from 180 to 190 g as shown in Table 4. The viscosity of the different formulations ranged from 15870 to 16696 cps at 10 rpm (Table 4), which shows the consistency of the formulations developed.

Results of the *in vitro* drug permeation study across artificial skin and rabbit skin are shown in Figs. 1 and 2, respectively. The formulations containing turpentine oil showed an increase in the cumulative amount of drug permeated as compared to gel containing no turpentine oil. From the results obtained, the presence of 3% turpentine oil seems to have the greatest effect on the permeation of IBF. The maximum flux was found to be 10.87 mg/cm^2/h across artificial skin and 17.26 mg/cm^2/h for P7 across rabbit abdominal skin, suggesting that increasing the concentration of turpentine oil causes an increase in the cumulative amount of drug permeated (Table 5). In fact, the permeability rate of IBF hydrogel containing 3% turpentine oil is significantly (one-way ANOVA, $p < 0.05$) greater than that of the other concentrations studied.

On the basis of the above results and discussion, we are well justified in concluding that turpentine oil causes an increase in permeation of IBF from gels due to increased disruption of the stratum corneum. The permeability increases as the concentration of turpentine oil increases. The primary mechansim of enhancing drug permeation might be disruption of the stratum corneum. Turpentine oil holds great potential for use as a natural penetration enhancer for hydrogel formulations.

Table 3: Physicochemical evaluation of different hydrogel formulations

Formulation code	pH	Drug content	Homogeneity	Skin irritation
P1	6.3	91.1%	+	Nil
P2	6.2	89.3%	+++	Nil
P3	6.7	91.4%	++	Nil
P4	6.3	95.7%	+++	Nil
P5	6.9	88.9%	+++	Nil
P6	6.4	89.6%	++	Nil
P7	6.9	89.2%	++	Nil

+++ Excellent ++ Good + Satisfactory

Table 4: Rheological data of different hydrogel formulations

Formulation code	Viscosity (cps) at 10 rpm	Spreadability g. cm/s	Extrudability (g)
P1	15870	5.57	190
P2	16045	5.94	188
P3	16187	6.16	185
P4	16354	6.80	180
P5	16467	6.41	184
P6	16696	6.53	185
P7	16564	6.52	183

3. Experimental

Samples of ibuprofen (IBF), carboxypolymethylene (CPM) and triethanolamine (TEA) were kindly donated by Leads Pharma Islamabad, the enhancer used was turpentine oil (Sigma Aldrich, Germany), and cellulose membrane, rabbit skin, ethanol (Merck Germany), potassium dihydrogenphosphate (Merck Germany), sodium hydroxide (Merck Germany). All chemicals were used without further purification.

3.1. Solubility study

Solubility was studied in three different solvents by adding access amount of the drug into each solvent and keeping the flasks on a shaking water bath for 24 h (Devi et al. 1999). After 24 h, all the solutions were analyzed spectrophotometrically at 223 nm taking respective solvent as blank, which was the absorption maxima for the drug and concentrations were determined.

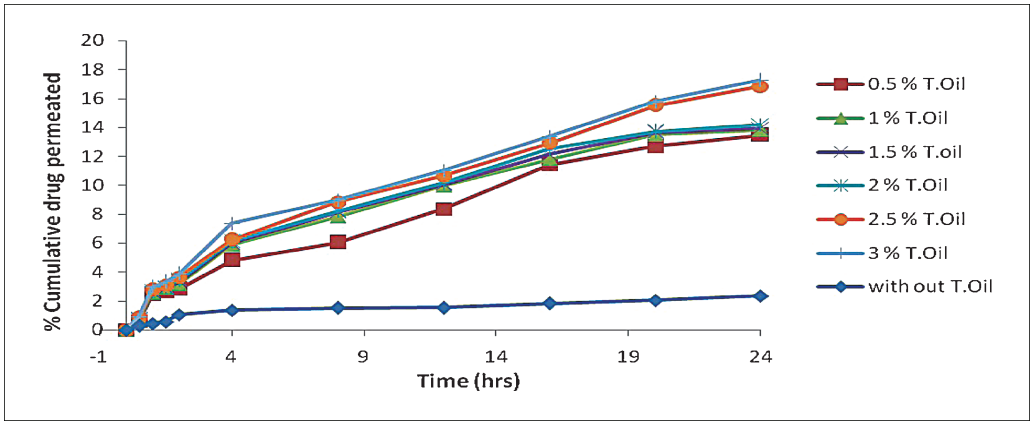

Fig. 2: Cummulative permeation of IBF gel across rabbit skin.

3.2. IBF (1% w/v) gel formulations

The required amount of gelling polymer (CPM) was accurately weighed and dispersed slowly in 50 ml of distilled water on magnetic stirrer at 400–600 rpm for 1 h. Weighed amount of IBF was dissolved in 20 ml of ethanol. The ethanolic drug solution was slowly added into previously prepared CPM suspension with continues stirring at 400–600 rpm till homogenous dispersion is achieved. Turpentine oil was incorporated in increasing concentration with. The final quantity was made up to 100 g with distilled water and 1 ml triethanolamine. The formulation is given in Table 1.

3.3. Evaluation of formulated gels

The formulated IBF gels were evaluated for drug contents and physically for pH, viscosity, spreadability, extrudability, smoothness and for appearance.

3.4. Drug content

A specific quantity (100 mg) of the developed hydrogel was dissolved in 100 ml of phosphate buffer pH 7.2. The volumetric flask containing hydrogel solution was shaken for 2 h on aq mechanical shaker in order to get complete solubility of drug. This solution was then filtered through membrane filter (pore size 0.45 μm). The absorbance of the sample was determined spectrophotometrically at 223 nm (Shimadzu 1601 UV-Visible Spectrophotometer) using Phosphate buffer pH 7.2 as a blank. The concentration of IBF was estimated from the regression equation of the calibration curve (Sera and Ramana 2006).

3.5. pH of hydrogel

The pH of the various hydrogel formulations was determined using a digital pH meter (Denver, USA) by dipping the pH meter electrode in gel samples. The measurement was carried out in triplicate and the average of the three readings was recorded.

Table 5: Permeation parameters of IBF from different hydrogels across artificial and rabbit skin

Formulation code	Artificial membrane flux ($mg/cm^2/h$) ± SD	Rabbit abdominal skin flux ($mg/cm^2/h$) ± SD
P1	2.15 ± 0.121	2.92 ± 0.174
P2	8.80 ± 0.779	11.43 ± 0.609
P3	8.61 ± 0.793	12.63 ± 0.630
P4	10.06 ± 0.847	12.99 ± 0.646
P5	10.23 ± 0.844	13.27 ± 0.659
P6	10.38 ± 0.844	14.15 ± 0.679
P7	11.19 ± 0.900	15.18 ± 0.749

3.6. Spreadability

Spreadability was determined by the wooden block and glass slide apparatus. This apparatus consists of a wooden block with a fixed glass slide and a pulley. About 20 g of hydrogel was placed on the fixed glass slide, and another (movable) glass slide with a pan attached to it, was placed over the fixed glass slide. Hydrogel was sandwiched between the two slides for 5 min. The time taken for the upper (movable) slide to separate completely from the fixed slide was noted (Gupta and Gound 1999).
Spreadability was determined using the following formula,

$$S = M.L/T$$

where,
S = Spreadability in g.cm/sec
M = Weight tied to upper slide
L = Length of glass slide
T = Time taken for the slides to separate completely from each other.

3.7. Homogeneity

All the hydrogels developed were tested for homogeneity by visual inspection. They were tested for their appearance and feel on application.

3.8. Viscosity

The viscosity of the formulated hydrogels was measured with a Brookfield viscometer using spindle S04. The spindle of the viscometer was rotated at 2.5, 4, 5 and 10 rpm. Samples were measured at 30 °C.

3.9. Extrudability

A closed collapsible tube containing hydrogel was pressed firmly at the crimped end, and the hydrogel was extruded until the pressure dissipated. The weight in grams required to extrude a 0.5 cm ribbon of hydrogel in 10 s was determined.

3.10. Skin irritation study

Skin irritation tests were performed on human volunteers. For each hydrogel, six volunteers were selected and 1.0 g of formulated hydrogel was applied to an area of 2 square inches (25.8 cm^2) on the back of hand. The volunteers were observed for erythema and edema for 48 h after application.

3.11. Preparation of rabbit skin

A number of animal models have been reported for performing *in vitro* permeation studies, e.g. rat (Catz and Friend 1990), hairless mouse (Catz and Friend 1990), rabbit (Hirvonen et al. 1993), shed snake skin (Buyuktimkin et al. 1995) and human cadaver skin (Roy et al. 1994). Although human cadaver skin may be the best choice, as a skin model for transdermal permeability experiments for transdermal therapeutic systems to be used in humans, it is not easily available. Hence in the present study, rabbit abdominal skin was used after removing the hair. Male healthy rabbits were anesthetized with chloroform inhalation and then sacrificed. The abdominal skin of the animal was shaved with the help of animal hair clipper and full thickness skin was surgically removed from each rabbit. The skin specimen was cut into appropriate size sections after carefully removing subcutaneous fat and washing with distilled water, then wrapped in aluminum foil and stored at −20 °C until it was used.

3.12. In vitro and ex vivo permeation study of gels

Permeation was studied in a Franz cell apparatus across both artificial skin and hairless rabbit skin. The receptor compartment was filled with pH 7.42 phosphate buffer. The temperature of the cell was maintained at $37 \pm 0.1\,^{\circ}C$ by continuous circulation of water through the cell jacket. The donor compartment was loaded with 1 g samples of gel. The receiving medium was stirred with a magnetic bar. A sample of volume 2 ml was withdrawn at different time intervals for 24 h. Each time, an equal volume of fresh receiving medium was added to replace the volume withdrawn. The samples were analyzed spectrophotometrically at 223 nm taking phosphate buffer pH 7.2 as a blank. The amount of the drug permeated per square centimetre at each time interval was calculated and plotted against time.

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