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Original Research Article

Preparation and physicochemical evaluation of transdermal aerosols containing ketoprofen

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Abstract

Purpose: To prepare transdermal ketoprofen metered-dose aerosol formulations containing menthol and isopropyl myristate (IPM) as penetration enhancers and to evaluate their physicochemical and permeation properties.

Methods: Selected ratios of ketoprofen, ethanol, polyvinylpyrrolidone K30 (PVP K30, anti-nucleant), and penetration enhancers were mixed and filled into aluminum aerosol cans with hydrofluoroalkane-134a as propellant. The physicochemical properties of the films, and their ex vivo permeation properties in rat skin were investigated over a 24-h period. Rat skin was exposed to a definite dose of each ketoprofen film to determine the quantity of drug in the receptor compartment over a 24-h period.

Results: All the transdermal formulations showed good integrity and film uniformity. Formulations with permeation enhancers presented higher rates of skin penetration compared to control formulations. Formulations containing 3% PVP and 5% IPM produced the highest permeation rates (J_{ss} , 85.35 $\mu\text{g}/\text{cm}^2/\text{h}$).

Conclusion: These results indicate that incorporation of antinucleant polymer and permeation enhancers in aerosols containing ketoprofen enhances aerosol stability and drug permeation through rat skin.

Keywords: Ketoprofen, Antinucleant, Permeation enhancer, Topical aerosol, Transdermal delivery

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INTRODUCTION

The therapeutic level of drugs after transdermal exposure has been extensively studied [1-3]. The stratum corneum is a major barrier to drug penetration through the skin. Since the lipid domain of stratum corneum is affected by chemical permeation enhancers, different chemical and biological enhancers that affect the lipid domain have been investigated [4-7]. Ketoprofen is a Non-Steroidal Anti-Inflammatory Drug (NSAID) which relieves inflammation-induced pain, as well as vascular headache and

dysmenorrhea. It is usually administered through the oral or rectal route [8]. Gastrointestinal complications such as ulcerations, abdominal burning sensation and pain, are some common side effects of ketoprofen [9]. To avoid these side effects, transdermal formulations, such as ketoprofen cream and ketoprofen ointment, have been developed for periodontal disease and osteopenia [10,11]. Percutaneous absorption of ketoprofen cream formulation and its effect on osteopenia have been studied [10-12]. Furthermore, the efficacies of different ointment bases and a new derivative of soy lecithin have

been investigated in the transdermal deliveries of drugs [13,14]. Indeed, investigations have been carried out on the effects of various topical formulations on different diseases, such as migraine, postoperative sore throat, and muscle soreness [15,16]. In recent years, transdermal metered-dose aerosols have been introduced as a new system of drug delivery to the skin. The advantage of transdermal aerosols over other topical routes of administration is the absence of any local irritation [17,18].

The purpose of this study was to prepare some transdermal aerosol formulations of ketoprofen and investigate their skin penetration capacities in the presence of penetration enhancers.

EXPERIMENTAL

Materials

Ketoprofen was received as a gift from Iran Hormone Co., Tehran, Iran. Pure ethanol (99.7-100%) and PVP K30 were purchased from Bidestan (Tehran, Iran) and Fulka, (Switzerland), respectively. In addition, 1,1,1,2-tetrafluoroethane (HFA 134a) was kindly gifted from INEOS Flour, UK. Methanol and IPM were provided by Pars Daru (Tehran, Iran) and Sepidaj (Tehran, Iran), respectively.

Formulation and preparation of metered-dose aerosol

Metered-dose aerosol solutions were formulated by dispersing ketoprofen (as a filmforming polymer, PVP) and an enhancer in pure ethanol (Table 1). The compounds were mixed with a magnetic stirrer overnight to solvate the polymer. Thereafter, aliquots of the solutions were dispensed into aerosol containers (Bespak Europe Ltd., UK). A 63- μ L valve was crimped on each container and the can was then filled with filtered HFA 134a. Ketoprofen concentrations were adjusted to 1.6-1.9 mg per puff, and pure ethanol was set at 1.0% (w/w). The latter allowed for reduction of negative effects on the vapor pressure and the release velocity of the liquefied propellant through the orifice. Each polymer film formation was tested by spraying it onto a clear plastic plate and allowing the solvent to evaporate.

Optical microscopy

The homogeneities of the solutions were observed using an optical microscope (Euromex, Netherlands) equipped with a camera system (Sony, Japan). The morphologies of the produced films were assessed before and after

the films were filled into the cans. For this purpose, the formulation was sprayed and photographed.

Table 1: Composition of transdermal metered-dose aerosol formulations

Formulation	PVP (%)	IPM (%)	Menthol (%)
F1	5	5	-
F2	7.5	7	-
F3	5	-	5
F4	7.5	-	5
F5	7.5	-	5
F6	5	-	-
F7	7.5	-	-
F8	3	5	-
F9	3	7	-
F10	3	-	2.5
F11	3	-	5
F12	3	-	-

The contents of ethanol (40%) and ketoprofen (5%) were the same in all the formulations. Each of the formulations was made up to 100% with HFA 134a

Preparation of skin membranes

The *in vitro* study was performed by using full-thickness skins of 8-week-old male rats. The experimental protocols were approved by Institutional Review Board of Faculty of Pharmacy, Tehran University of Medical Sciences (ref no. 5074). The animals were handled according to EU Directive 2010/63/EU on the handling of animals used for scientific purposes [19]. Using a surgical blade, the skins were isolated by separating the underlying subcutaneous fat and connective tissue. The skins were kept frozen in a freezer at -20 °C until used. Prior to use, they were they were transferred to a room temperature setting.

Drug permeation studies

The skins were clamped in vertical Franz diffusion cells with a donor surface area of 3.8 cm² per skin, and maintained in a water bath at 37 °C [20]. Phosphate-buffered saline (PBS, 23 mL, pH 7.4) was used to fill the receptor compartment. Temperature was set at 37 °C throughout the 24-h experiment using a water bath. At the beginning of the diffusion experiments, 5 puffs of the ketoprofen

formulation were applied to the exposed area of the skin. Each sample (1 ml) was collected from the receptor compartment at 10 min, and at 1, 2, 4, 6, 8 and 24 h post-dosing, and replaced with 1 ml of fresh PBS.

High-performance liquid chromatography (HPLC)

HPLC was employed for the determination of drug concentration in each sample. Separation was carried out with 4:17 (v/v) ratio of phosphoric acid:methanol as a mobile phase at a flow rate of 1.2 mL/min in an EC 250/4.6 Nucleodur 100-S C18ec column. The column temperature was set at 40 °C and injection volume was 100 µL. The peak area of chromatogram obtained at 256 nm was taken as reference for determination of drug concentrations in the samples.

Data computation

The cumulative amounts of the drug that penetrated per unit surface area (µg/cm²) were analyzed as a function of time (h). The following formula was applied to calculate the *in vitro* skin permeation rate or steady state flux (J_{ss}).

$$J_{ss} = \Delta M / A \Delta t \dots\dots\dots (1)$$

where ΔM is the amount of ketoprofen that penetrated through the skin within a defined time Δt , and A is the diffusional area.

The cumulative amount of the absorbed drug in 24 h was also measured. The effect of each penetration enhancer was represented by an

Enhancement Ratio (ER), which was calculated as shown in Equation 2 below.

$$ER = J_{ss}F / J_{ss}C \dots\dots\dots (2)$$

where $J_{ss}F$ is the skin permeation of formulation with enhancer, and $J_{ss}C$ is the skin permeation of formulation without enhancer.

Statistical analysis

The data are presented as mean ± standard deviation (SD). Differences between results obtained for formulations containing various excipients were statistically analyzed using Student’s t-test with the aid of SPSS 16.0 statistical software. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Characteristics of the formulations

Tough films with smooth surfaces were produced by all the formulations. However, when examined under the microscope, all the films had some fine air bubbles.

The cumulative amounts of ketoprofen that permeated from the films through the rat skin over 24 h were calculated by HPLC. The cumulative amounts of the drug release for all formulations with enhancers were significantly ($p < 0.05$) higher than for those without the permeation enhancers (Figures 1 and 2).

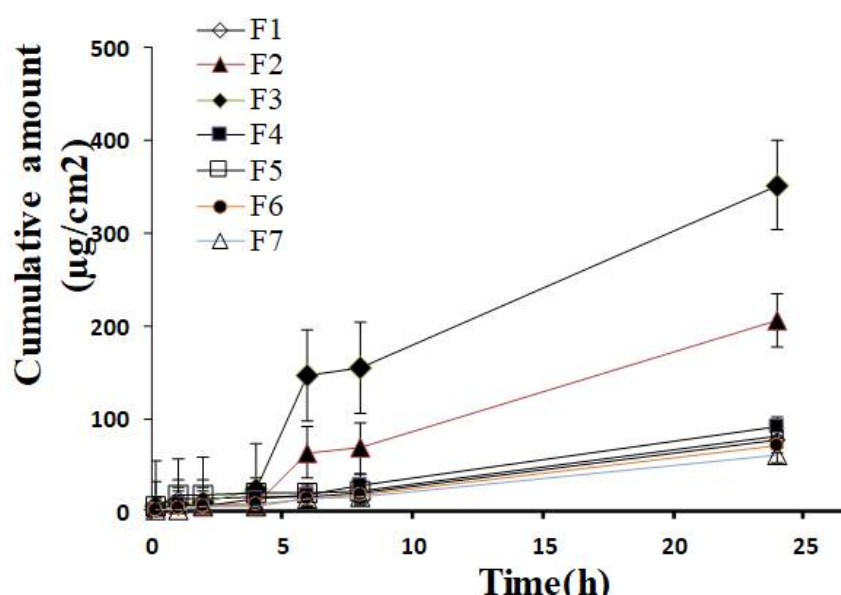


Figure 1: Permeation profile (mean ± SD) of formulations prepared with 5 and 7.5% PVP (n = 3)

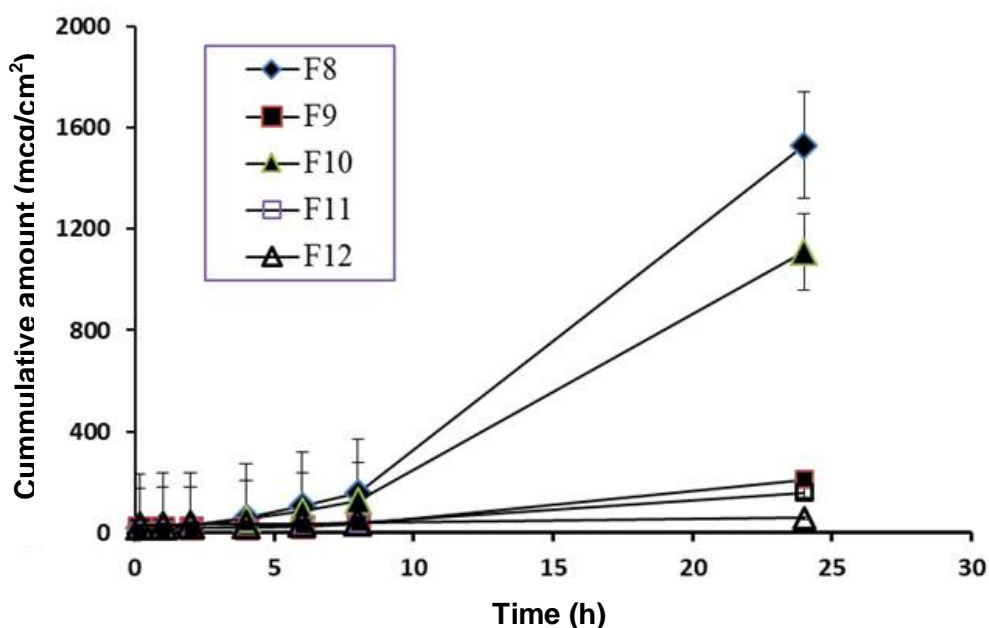


Figure 2: Permeation profile (mean \pm SD) of formulations prepared with 3% PVP (n = 3)

Table 2: Permeation rate (J_{ss}) and enhancement ratio (ER) of formulations prepared with 5 and 7.5% PVP

Formulation	J_{ss} ($\mu\text{g}/\text{cm}^2/\text{h}$)	ER
F1	3.64 \pm 0.017	1.10 \pm 0.026
F2	8.71 \pm 0.144	3.24 \pm 0.033
F3	12.39 \pm 0.104	3.75 \pm 0.046
F4	3.87 \pm 0.139	1.44 \pm 0.035
F5	3.66 \pm 0.009	1.37 \pm 0.018
F6	3.30 \pm 0.070	-
F7	2.68 \pm 0.030	-
F8	85.36 \pm 0.663	79.38 \pm 0.683
F9	10.71 \pm 0.549	9.96 \pm 0.566
F10	61.14 \pm 0.786	56.86 \pm 0.81
F11	6.97 \pm 0.358	6.48 \pm 0.369
F12	1.08 \pm 0.017	-

In the formulations with 5% PVP, F3 showed a significantly ($p < 0.05$) higher permeation rate than F1 (Table 2). Formulations with 7.5% PVP increased the permeation rate approximately as much as those of the formulations containing 5% PVP.

Drug release, in the presence of lower amounts of PVP, were evaluated for formulations F8 to F12, with PVP concentration at 3%. As can be seen in Table 2, formulations with 3% PVP produced significantly ($p < 0.05$) higher permeation than those with 5.0 - 7.5% PVP.

Among all the formulations, F8 which contained 5% IPM and 3% PVP had the highest permeation rate. This formulation showed a drastic rise in the

amount of ketoprofen that permeated through the rat skin.

DISCUSSION

PVP is a well-known inhibitor of drug crystallization in pharmaceutical formulations. Many researchers have studied the inhibition mechanism of PVP in aqueous suspensions or solid dispersions [21, 22]. Amorphous drugs are in a high energy state that causes increased solubility and consequently a high thermodynamic activity and penetration rate [22]. At high concentrations of PVP, this effect may be reduced because of the interaction between the drug and PVP [23]. Thus, drug release in the presence of lower amounts of PVP was studied on formulations F8 to F12, where PVP concentration was lowered to 3%. This modification in the formulations resulted in a drastic rise in the amount of drug that permeated through the rat skin. Indeed, when the penetration enhancers were applied at the concentrations of 5% (for IPM) and 2.5% (for menthol), steady state flux reached values of 85.35 and 61.13 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively. Therefore, in these series of studies, an optimal level of film former was identified so as to ensure the highest drug penetration rate for each enhancer.

The presence of IPM or menthol in the films increased the flux of the drug, unlike in films without the enhancers. Calculation of ER for comparing the permeation rates of the drug in

the presence of the penetration enhancers revealed a considerable increase in the flux of the drug through the rat skin. The increased drug flux implies that the studied formulation would provide durable therapeutic effects. This enhancement in drug activity can be due to a number of factors, such as the kinetics of the enhancer release from the film, and the interaction between the enhancers and other components of the film. These factors may induce varied effects depending on the amounts of polymers and enhancers [23]. Moreover, the enhancing effect depends on the composition of the formulation and the physicochemical features of drugs [24].

Terpenes have been reported to increase the permeations of various groups of drugs, and amongst the terpenes, menthol enhances the penetrations of hydrophilic drugs [25,26]. The mechanisms involved in enhancement of drug penetration by terpenes include perturbations in the skin layer and lipid domains [27]. Various analogues of fatty acids have been studied as penetration enhancers [28]. Fatty acid esters generally affect drug permeation by increasing the fluidity of intracellular lipids [29]. In the present study, the penetration enhancers used, which are compatible with ethanol and HFA 134a, were found to increase the penetration rate of ketoprofen after 8 h. This suggests that within 8 h, the enhancers produced optimum effects by positioning themselves between the phospholipid bilayers in the stratum corneum.

CONCLUSION

The improved duration of release of high concentration of ketoprofen seen in this study is made possible by the formation of satisfactory films with the aid of PVP as an anti-nucleant. The films containing 5% IPM and 3% PVP is capable of enhancing skin permeation of ketoprofen up to 79.38-fold in rats when compared with formulations without an enhancer. Thus, the formulations have a potential for transdermal delivery of ketoprofen in therapeutics.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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