INFLUENCE OF OLEIC ACID ON THE IN VITRO PENETRATION OF DICLOFENAC SODIUM GEL

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INTRODUCTION

Diclofenac sodium (DS) is a nonsteroidal antiinflammatory drugs (NSAIDs), that inhibits cyclooxygenase-2 enzyme (COX-2). It has first pass metabolism by 40-50%, because of its short biological half-life, the drug has to be given frequently (Ganiswara, 2005). Dose of DS in gel is 1% (Sweetman, 2007). In peroral use, it may cause the risk of gastrointestinal bleeding, cardiovascular, hypersensitivity reactions and disorders of the central nervous (Katzung, 2002, Chuasuwan, et al., 2008).

DS does not penetrate well through skin and cannot reach the effective concentration at the site of action after transdermal application (Mohammed, 2001; Ozguney, et al., 2006). DS partition coefficient in n-octanol-buffer aqua (log P) was 1.1 (Chuasuwan, 2008). Lipophilic nature of the stratum corneum and the hydrophilic nature of the underlying tissues showed that the drug will penetrate the skin should have an optimal balance between the lipophilic and hydrophilic properties. In general, the skin is more permeable to the material which has a partition coefficient (P) in octanol-water between 10-1000 (Michniac-Kohn et al., 2005).

Oleic acid enhances the penetration of drugs into the skin by increasing the fluidity of the stratum corneum lipids through the establishment of channels (water channels) (Fang , et al , 2003)

This study aimed to know the influence of Oleic Acid (OA) in several concentrations (1%, 3% and 5%) as penetration enhancer on the in vitro penetration of topical DS gel. Furthermore, the physical properties of the topical preparation (organoleptic, pH and viscosity), and DS penetration percutaneously through rat skin . Then observed that in spite of DS assay and penetrate through the rat skin at certain intervals and observed by means of HPLC.

MATERIALS AND METHODS

Materials

Diclofenac sodium was obtained as a gift sample from PT. Kimia Farma, Carbopol 940, Propylene glycol, Triethanolamine, Potassium Chloride, Potasium Phosphate Dibasic, Sodium Phosphate Dibasic, Sodium Chloride, Glacial Acetic Acid p.a, Methanol p.a, distilled water, distilled water pro HPLC

Instruments

UV Visible Spectrophotometer Hitachi UV1800, HPLC Shimadzu, Dissolution Tester Pharmeq, diffusion cell, pH meter Denver, Viscometer Rion VT-04E, filter holder, Millipore Membrane Filters 0,45µm,

Preparation of Topical Formulation

Gel was prepared with Carbopol 940, triethanolamine**,** propylene glycol, oleic acid and distilled water (Table 1) using 2 mixtures. Mixture I was obtained by dispersing Carbopol 940 in a mixture of distilled water and neutralized by the addition of triethanolamine. Mixture II was obtained by dissolving DS in a mixture of propylene glycol and distilled water. After complete hydration of Carbopol 940, mixture II was added drop by drop to mixture I by stirring manually. Oleic acid was added to mixture by stirring manually. The resulting gel stored at room temperature for 24 h study prior to use.

Constituents	Concentration (%)			
	F0	F1	F2	F3
DS	1	1	1	1
Carbopol 940	0.6	0.6	0.6	0.6
Triethanolamine	qs	qs	qs	qs
Propylene glycol	30	30	30	30
Oleic acid	-	1	3	5
Distilled water to	100	100	100	100

Table 1. Composition (% w/w) of DS gels

Evaluation of Gels

Visual appearance

The prepared gels were visually inspected for consistency, color, and transparency.

pH of the gels

The pH of gel was determined after diluting and dispersing it in distilled water (10% w/v). All the measurements were made in triplicate and mean calculated.

Viscosity Determination

Gel viscosity measurements were evaluated using a viscosimeter Rion VT-04. All viscosity measurements were performed in triplicate and mean calculated

Drug Content Uniformity

A series of working standard solution containing (0.4-15.3 µg/ml) of DS were prepared. A 20 µl a liquot of the solution was injected on to the column in a duplicate and the chromatograms were recorded

Exactly 0.25 g gel was completely dispersed in distilled water to make final volume 25 mL by subjecting it to stirring for 5 min. The dispersion was than filtered to remove the undissolved residue. Exactly 1 mL of the filtrate was diluted to 10 mL. An unloaded gel was also subjected to a similar determination to observe the effect of excipients on the absorbance. Using the standard curve of DS in distilled water, the drug content in gel was finally estimated by HPLC.

In Vitro penetration studies

The abdominal hair of male Wistar rats, weighing 130-140 g was removed carefully, without damaging the underlying skin, using clippers Full-thickness skin was excised from the abdomen under ether anesthesia.(Hadgraft and Ridout, 1987; Miller et al., 1993). Adhering subcutaneous fat phases were disposed from the dermal surface. The samples were then allowed to diffusion cell, with the stratum corneum facing the donor separate into two phases and the concentration of DS in compartment.DS penetration rates through rat skin were measured using a system of Dissolution Tester-diffusion cells. with an available diffusion area of 1.77 cm_2. In this study, 500 ml of phosphate buffer saline (PBS) solution (pH 7.4) was used as the receptor medium and 2 g of the test gel was placed on the donor side. The receptor medium was kept at 37 ^oC and stirred at 50 rpm. At predetermined time intervals, 5-ml samples were taken from the receptor compartment, for an 8-h period, and replaced by the same volume of fresh PBS to maintain a constant volume. DS was determined by HPLC. DS steady-state flux, *J*, was estimated from the slope of the straight line portion of the cumulative amount of drug absorbed against time profiles.

Analytical procedure

The amount of DS in permeation samples was determined using HPLC apparatus (Shimadzu). Elution was carried out at room temperature with a mobile phase consisting of methanol–water (9:1,v/v) adjusted to pH 2.2 with glacial acetic acid; the flow-rate was 1 ml /min. Detection was at 276 nm.

RESULTS AND DISCUSSIONS

Evaluations of Gels

Visual appearance

The prepared gels consistency, color, smell, and transparency are reported in Table 2

Table 2. Visual appearances of SD Gels

For-mula	Visual appearances		
	Consistency	Color	Trans-parency
0	Gel	White	Yes
1	Emulgel	Yellowish white	No
2	Emulgel	Yellowish white	No
3	Emulgel	Yellowish white	No

pH Determination

The transport of DS across the abdominal rat skin was investigated from a DS gel dosage form. The pH value of the vehicle, the drug solubility in the vehicle and the viscosity of the gel are three important factors to consider in the evaluation of drug penetration from a gel dosage form across the skin (Ho et al., 1994). Therefore, carbopol gels were adjusted to pH 7 to minimize any pH effect. Oleic acid is acidic and has a pH of 4.4 (Rowe , 2006) . On the addition of oleic acid at a concentration of 5 % pH value becomes smaller than the other formulas

Viscosity Determination

The viscosity of the gel matrix may play an important role in controlling the release of the drug into the receptor compartment when the drug diffusion through the gel matrix is a rate determining step. However, formulations as they are contents OA caused a decrease in the viscosity. The viscosity of the gel without OA (F0) was the highest, whereas that of the gel containing 5% OA was the lowest, because the amount of water used to swell Carbopol decrease with the addition of OA, so it become less swelling. The pH and viscosity readings are reported in Table 3.

Drug Content Uniformity

Calibration curves were constructed in the range of concentrations of 0.4 - 15.3 µg/ml for DS. The linear regression equation obtained was y = 42971.13x + 481.72, with a correlation coefficient (r) = 0.9998. The calibration curve are presented in Fig 1.

Table 3. pH and Viscosity of DS gels

Formula	pH ± SD	Viscosity (dPas)± SD
F0	7,10 ± 0,03	123,33 ± 2,89
F1	7,10 ± 0,01	106,27 ± 2,89
F2	7,02 ± 0,08	97,33 ± 2,52
F3	6,69 ± 0,04	78,33 ± 2,89

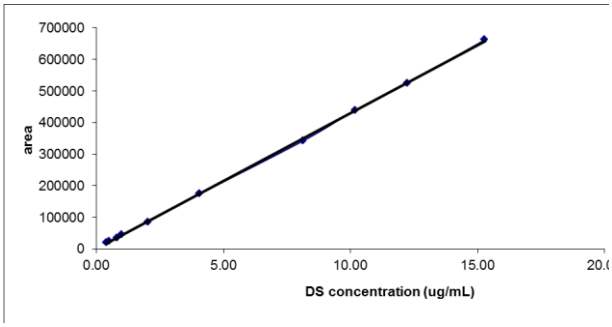

Figure 1. Calibration curve of diclofenac sodium by HPLC analysis

The drug content uniformity of all formula are reported in Table 4.

Table 4. Drug Content Uniformity

Formula	Drug Content (%) ± SD	RSD (%)
F0	95.29 ± 0.91	0.95
F1	94.48 ± 0.32	0.33
F2	97.16 ± 0,71	0.73
F3	96.36 ± 0.94	0.98

It was observed that the drug content of all formulas was fulfilled the requirement, between 80-110% and RSD less than 6%

In Vitro penetration studies

With respect to drug permeation through the skin from vehicles, a drug should first diffuse out from the vehicle to the skin. Thus, the influence of OA on the penetration of DS from the carbopol gels through abdomen rat skin was examined. The penetration profiles of DS from these gels through the abdomen rat skin are presented in Fig. 2. The penetration profiles of F1(OA 1%) was almost similar to that from the gel F2 (OA 3%), but F2 has the higher penetration flux of DS. F3(OA 5%) has more lower penetration flux of DS than F1 and F2. F0 (OA 0%) has the lowest fluxes. The mean maximum fluxes obtained from the penetration of DS from abdomen rat skin are presented in Fig. 3.

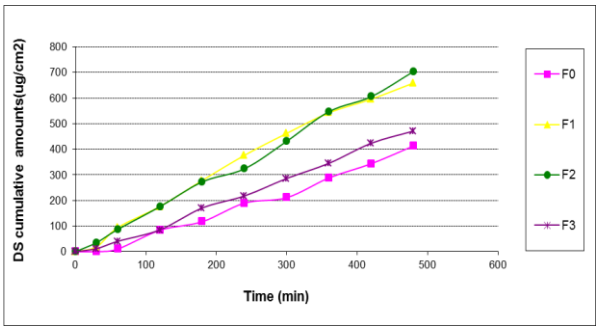

Figure 2. Penetration profiles showing the effect of different grades of Oleic acid on the penetration of diclofenac sodium

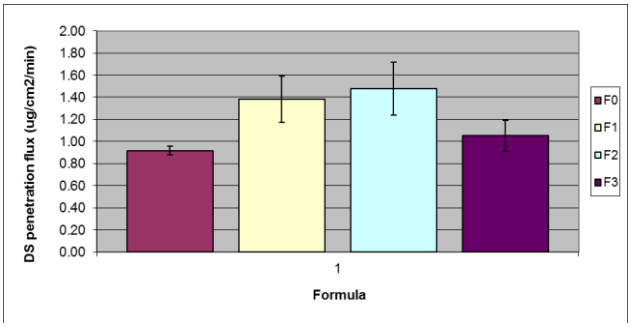

Figure 3. Mean maximum fluxes obtained from the penetration of diclofenac sodium from abdomen rat skin (n = 3)

In the formula 1 and 2 , which were DS penetration more higher than the formula 0 and 3, contained OA 1% and 3 % in the DS gel. OA helped DS penetration into the skin. Mechanism of OA as penetration enhancers was increasing the fluidity of the stratum corneum lipids through the water channels (Fang, et al , 2003), so that the DS permeation was more easier to penetrate the stratum corneum . In the formula 3 contains OA 5% caused skin more lipophilic, DS will be retained longer on the skin and it was difficult to penetrate the stratum corneum. In the formula 0, DS largely dissolved in a gel matrix containing propylene glycol. Improved solubility of the active ingredient in the gel matrix was usually followed by a decrease in partition coefficient, so, the ability of DS to penetrate the stratum corneum was also decrease (Arellano et al , 1998) .

CONCLUSION

The results presented in this article showed, the penetration enhancer action of OA in the penetration of DS across abdomen rat skin from carbopol gels was increasing the fluidity of the stratum corneum lipids through the water channels. Carbopol gels contained OA 1% and 3% provide the similar penetration flux and more higher than OA 0% and 5%.

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