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Research Article

EFFECT OF PENETRATION ENHANCER ON THE *IN VITRO EX VIVO* PERMEATION OF DICLOFENAC GEL

MUKESH SHINDE[1]*, VIKRAM GHARGE[1], SHRIKANT PIMPLE[2], MUKUND GURJAR[2], MAHESH SHAH[2]

[1]Zuventus Healthcare Ltd, Pune - 411 026, Maharashtra, India. [2]Emcure Pharmaceutical Ltd., Emcure House, Pune - 411 026, Maharashtra, India. Email: mukesh.shinde@zuventus.com

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ABSTRACT

Objective: The objective of this study was to compare and evaluate in vitro ex vivo transdermal potential of the diclofenac sodium (DS) gel by different permeation enhancers using Franz-type diffusion cells.

Methods: DS gel was prepared with carbapol, dimethyl sulfoxide, oleic acid (OA), and menthol with 1% w/w concentration each as the penetration enhancer. The in vitro, ex vivo permeability were determined using cellophane membrane and abdominal rat skin. Steady-state flux, permeability coefficient (kp), diffusion coefficient, and lag time were calculated.

Results: Menthol, at the concentration of 1% w/w, shows maximum kp, diffusion coefficient permeation enhancement effect with an enhancement ratio 40%, as compared to dimethyl sulfoxide and OA.

Conclusion: This finding predicts that DS gel 1% w/w containing menthol 1% w/w can possibly deliver therapeutically relevant dose of DS.

Keywords: Diclofenac sodium gel, Menthol, Penetration enhancer, Permeation coefficient.

INTRODUCTION

Transdermal drug delivery gives many important advantages such as it is easy for application, protect the active compound from gastric and enzymatic degradation, simple to terminate the therapy if undesired side effect occurs [1]. Skin is a natural barrier, and only few drugs can penetrate through it easily in sufficient quantity [2]. Diclofenac is a strong nonsteroidal anti-inflammatory drug (NSAID) clinically used for the management of acute conditions of inflammation and pain, musculoskeletal disorders, and arthritis. Although diclofenac is a relatively safe and tolerable NSAID, the clinical use of diclofenac has been often limited because of its potential to cause irritation and ulceration of the gastrointestinal mucosa after oral administration [3]. NSAIDs are known to inhibit cyclo-oxygenase-2 at the inflammation sites, but most of them also inhibit gastric mucous cyclo-oxygenase-1, which is associated with gastrointestinal damage [4]. The oral formulation of diclofenac is accompanied with the above mentioned adverse effects as well as high degree of hepatic first-pass metabolism and short biological half-life [5,6]. Therefore, a transdermal delivery system has been attempted to overcome these disadvantages and maintain relatively consistent plasma levels for long-term therapy from a single dose [7]. However, because few reports on the transdermal delivery system for diclofenac have not been very satisfactory [8,9], the use of an optimal chemical enhancer may be the key to a more successful transdermal delivery system for diclofenac sodium (DS). The relative impermeability of the stratum corneum (SC) provides the principal resistance to percutaneous absorption of most drugs. The key barrier to transdermal drug delivery is the outermost layer of the skin, the SC. The primary approach to overcome skin resistance to drug penetration is the skillful selection of penetration enhancers, substances that facilitate penetration by reversibly altering the structure of the skin [10,11].

In this study, DS was formulated as a gel because of the favorable properties of this type of a topical formulation. As gel tends to be smooth, elegant, and non-greasy, produce cooling effects and utilize better drug release as compared to other semi-solid formulation. Three types of penetration enhancers were tested, the lipid disrupting agent oleic acid (OA) that increases the fluidity of SC lipids [12]; the aprotic solvent dimethyl sulfoxide (DMSO) that denature proteins, change the intercellular keratin conformation, and interact with the intercellular lipid to distort their geometry [13]; the terpan menthol that modify the solvent nature of the SC and usually decrease the lag time for permeation [14]. In this paper, the influence of penetration enhancer on the in vitro penetration of DS through a cellophane membrane and rat skin from carbopol gels was investigated. The permeability effect of penetration enhancer on the percutaneous penetration of DS was also evaluated.

METHODS

DS, carbopol 940, DMSO, OA, menthol, disodium hydrogen phosphate, triethanolamine, and sodium hydroxide were used from Zuventus R and D center, Pune. All other reagents and solvents used were pharmaceutical grade.

Gel formulations

Diclofenac gel (1% w/w) was prepared using carbopol 940 (1% w/w), triethanolamine (0.4% w/w), ethanol and distilled water (Table 1). DS was dispersed in mixture of 24.1% w/w distilled water and 18.72% w/w ethanol, which was added drop-wise into hydrated

Table 1: Contents (% w/w) of diclofenac gel formulation

S. no.	Ingredients	DS-A*	DS-B*	DS-C*	DS-D*
1	DS	1.0	1.0	1.0	1.0
2	Carbopol 940	1.0	1.0	1.0	1.0
3	Triethanolamine	0.4	0.4	0.4	0.4
4	Ethanol	24.5	24.5	24.5	24.5
5	DMSO	0.0	1.0	0.0	0.0
6	OA	0.0	0.0	1.0	0.0
7	Menthol	0.0	0.0	0.0	1.0
8	Distilled water	74.1	74.1	74.1	74.1
Total		100	100	100	100

*DS-A: Control gel, DS-B: With DMSO, DS-C: With oleic acid, DS-D: With menthol, DS: Diclofenac sodium, DMSO: Dimethyl sulphoxide, OA: Oleic acid

carbopol 940 containing 6.25% w/w ethanol and 49.3% w/w distilled water, with string until to form gel. After gel formed immediately add different permeation enhancer as shown in Table 1.

Characterization of DS gel

The prepared DS gels were inspected visually for their pH, viscosity, spreadability, homogeneity, consistency, drug content, in vitro drug release [15].

pH

The pH was checked using a pH meter (mettler toledo seven easy), which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 minutes priors to taking a reading at room temperature.

Viscosity

The measurement of viscosity of the prepared gel was done with a Brookfield viscometer. The gels were rotated at 20 and 30 rpm using spindle no. 64. At each speed, the corresponding dial reading was noted.

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load, lesser the time taken for separation of two slides, better the spreadability.

It is calculated using the formula (1):

$$S = \frac{M \times L}{T} \quad (1)$$

Where, M = Weight tied to the upper slide
L = Length of glass slides
T = Time taken to separate the slides

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Consistency

Measurement of consistency of the gels was carried out by dropping a cone attached to a holding rod from a fix distance of 10 cm in such way that it falls in the center of a glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance traveled by the cone after 10 second was noted.

Drug content uniformity

Drug content uniformity was determined by ultraviolet spectrophotometer. 1 g of formulated gel was taken from a different region, of gel and dissolved in phosphate buffer pH 7.4. The absorbance was taken at 277 nm using (UV-visible V 530 Jasco Japan).

***In vitro* permeability studies through cellophane membrane**

In vitro diffusion studies were carried out using modified Franz diffusion cell (FDC 06/orchid scientifics and innovative India Pvt. Ltd., Nasik, India) with the diffusional area of 4.67 cm^2. Cellophane membrane (Sartorius, Goettingen, Germany) was sandwiched between the lower cell reservoir and the glass cell-top which was secured in place with a pinch clamp. The receiving compartment was filled with 28 mL phosphate buffer pH 7.4, which was maintained at 37±0.5°C by a water bath circulator and a jacket surrounding the cell, resulting in a membrane-surface temperature was 37°C. A Teflon coated magnetic bar continuously stirred the receiving medium to avoid diffusion layer effects. 1 g sample was placed evenly on the surface of the membrane in the donor compartment that was sealed with aluminum foil to prevent evaporation. At predetermined time intervals, 1 mL samples were taken from the receptor compartment, for a 6 hrs period, and replaced by the same volume of fresh buffer to maintain a constant volume. DS was assayed spectrophotometrically [16].

***In vitro* permeability studies through skin**

The abdominal hair of male Wistar rats, weighing 200-250 g, was removed carefully, without damaging the underlying skin, using electric clippers. Full-thickness skin was excised from the abdomen under ether anesthesia adhering subcutaneous fat, and other extraneous tissues were trimmed if necessary from the dermal surface. Then, the excised skin was equilibrated in phosphate buffer (pH 7.4) for 1 hr before being mounted on the FDC, with the SC facing the donor compartment. At predetermined time intervals, 1mL samples were taken from the receptor compartment, for an 6 hrs period, and replaced by the same volume of fresh buffer to maintain a constant volume. DS was assayed spectrophotometrically [17].

Calculating flux and permeability coefficient (kp)

Flux (J) is the amount of permeant (molecular species moving through or into tissue/membrane) passing through the membrane in time. It is given in units of mass/area/time or units of radioactivity/area/time. But if the permeant was applied infinite dose. The flux J calculated using the formula (2):

$$J = \frac{Q}{A \times t} \quad (2)$$

Where Q is the quantity of compound traversing the membrane in time t, and A is the area of exposed membrane in cm^2. Units of flux are quantity/cm^2/minutes.

Steady state flux (Jss) is the amount of permeant crossing the membrane at a constant rate; this occurs after the lag phase when drug was permeating over the time period continually. When the amounts measured at successive sampling intervals are not significantly different, this is considered steady state by formula (3)

$$Jss = \frac{Q}{A \times t} \quad (3)$$

Where Q is the quantity of compound transported through the membrane in time t, and A is the area of exposed membrane in cm^2. The unit of Jss is quantity/(cm^2 • hr).

If the amount of permeant applied to the membrane was an infinite dose, then the kp can be calculated from the relationship by following formula (4)

$$Kp = \frac{Q}{\left[A \times t \times (Co - Ci)\right]} \quad (4)$$

Where Q is the quantity of compound transported through the membrane in time t (minute), Co and Ci are the concentrations of the compound on the outer side (donor side) and the inner side (receptor side) of the membrane respectively, and A is the area of exposed membrane in cm^2. Usually, Co can be simplified as the donor concentration and Ci as 0. The units of Kp are cm/minute or cm/hrs [18-20].

RESULTS AND DISCUSSION

The transport behavior of DS across the cellulose membrane or abdominal rat skin was investigated from a gel dosage form prepared by gelling a solvent mixture of water and ethanol with carbopol (Table 1). Theoretically, the pH value of the vehicle, the drug solubility in the vehicle and the viscosity of the gel matrix are three important factors to consider in the evaluation of drug penetration from a gel dosage form across the membrane or the skin, therefore carbopol gels were adjusted to pH 5.62-5.70 to minimize any pH effect (Table 2).

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. The release

Table 2: Characterization, drug release kinetics of DS gel formulations

Gel	Homogeneity	Consistency (mm)	Spreadability (g. cm/second)	Viscosity (Cps)	pH	Drug content (%)
DS-A	++	7.50	32.84±1.19	4256±1.15	5.62±0.85	98.18±0.58
DS-B	++	7.50	28.26±1.47	3945±1.48	5.64±0.75	97.15±0.47
DS-C	++	7.50	29.35±1.24	4127±1.50	5.63±0.25	96.18±0.59
DS-D	++	7.50	39.28±1.25	4025±1.47	5.69±0.48	98.25±0.25

Excellent +++, Good ++, Satisfactory + ; *Data given as mean±SD (n=3), DS: Diclofenac sodium, SD: Standard deviation

profiles of the formulated gels followed matrix diffusion kinetics [21] confirming that DS was fully dissolved in the gel and, thus, the membrane used has no significant effect on the release of the drug (Tables 3 and 4).

The results for spreadability data indicate that the gel was easily spreadable with a small amount of shear. Consistency reflects the capacity of the gel to get ejected in uniform and desired quantity when the tube is squeezed. Consistency in terms of distance travelled by the cone was 7.5 mm. The gel formulations were homogeneous in texture and fell. The results show that there was no significant difference between the viscosities of the gel formulations with the penetration enhancer and that of the controlled gel (DS-A).

The effect of three penetration enhancers (DMSO, OA, and menthol) at 1% w/w concentrations, each on the permeability and release characteristic. The presence of penetration enhancers may change the thermodynamic activity of the drug DS in the vehicle and consequently alter its permeability. To sort out these two different effects, the formulations containing penetration enhancers were first evaluated on cellophane membrane and then on rat skin. In this way, we could detect possible negative interaction between the base gel and the penetration enhancers shows the permeation profiles. Jss, kp, diffusion coefficient lag time, and release rate were studied.

The effects of penetration enhancers were tested on rat skin as a barrier to assess which interaction with the SC is most favorable to DS penetration. The permeability profiles obtained are shown in Fig. 1-4. Tables 4 and 5 report the calculated Jss, kp, diffusion coefficient, and lag time from cellophane membrane and rat skin membrane.

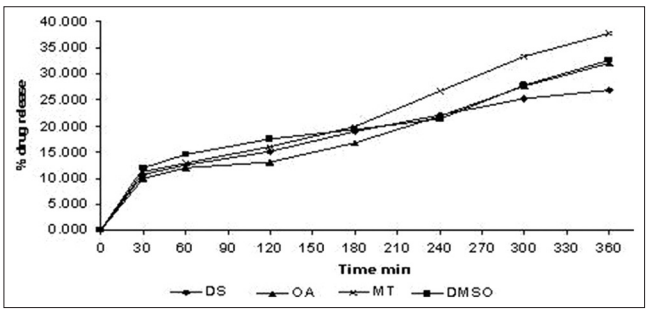

Fig. 1: Average % release of diclofenac sodium gel with a permeation enhancer through cellophane membrane

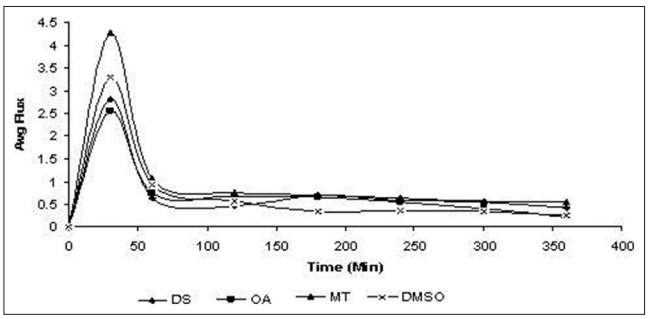

Fig. 2: Average flux of diclofenac sodium gel with a permeation enhancer through cellophane membrane

Table 3: Kinetic models for the formulation of the DS with a permeation enhancer through cellophane membrane

Kinetic model	Parameter	Formulation			
		DS-A	DS-B	DS-C	DS-D
Zero order	R	0.8615	0.8237	0.9452	0.9188
	K	0.0770	0.0875	0.0832	0.1047
	t-Test	4.156	3.558	7.093	5.702
First order	R	0.8981	0.8735	0.9617	0.9521
	K	−0.0009	−0.0010	−0.0009	−0.0012
	t-Test	5.003	4.393	8.593	7.623
Matrix	R	0.99	0.9874	0.9655	0.9825
	K	1.2609	1.4393	1.3353	1.6954
	t-Test	17.170	15.268	9.078	12.909
Peppas	R	0.9737	0.9811	0.9445	0.9567
	K	2.1003	2.7820	1.3244	2.1945
	t-Test	10.474	12.420	7.042	8.056
Hixon Crowell	R	0.8868	0.8581	0.9571	0.9426
	K	−0.0003	−0.0003	−0.0003	−0.0004
	t-Test	4.700	4.094	8.088	6.916

DS-A: Control gel, DS-B: With DMSO, DS-C: With oleic acid, DS-D: With menthol, DS: Diclofenac sodium

Table 4: Kinetic models for the formulation of the DS with the penetration enhancer through rat skin

Kinetic model	Parameter	Formulation			
		DS-A	DS-B	DS-C	DS-D
Zero order	R	0.8308	0.8362	0.9316	0.9456
	K	0.0880	0.0975	0.0936	0.1115
	t-Test	3.657	3.734	6.280	7.140
First order	R	0.8788	0.8879	0.9553	0.9697
	K	−0.0010	−0.0011	−0.0011	1.7922
	t-Test	4.512	4.729	7.912	9.723
Matrix	R	0.99	0.9727	0.9699	0.9748
	K	1.4476	1.5986	1.5071	1.7922
	t-Test	17.757	10.277	9.755	10.706
Peppas	R	0.9826	0.9545	0.9392	0.9561
	K	2.6451	3.2390	1.8265	1.8014
	t-Test	12.946	7.838	6.702	7.995
Hixon Crowell	R	0.8640	0.8726	0.9487	0.9635
	K	−0.0003	−0.0004	−0.0003	−0.0004
	t-Test	4.204	4.376	7.348	8.811

DS-A: Control gel, DS-B: With DMSO, DS-C: With oleic acid, DS-D: With menthol, DS: Diclofenac sodium

Table 5: DS with permeation enhancer permeation parameter through cellophane membrane

Enhancer	Gel code	Steady state flux (μg/cm^2/h)	Kp (×10^3) (cm/h)	Diffusion coefficient 10^{-8} cm×h^{-1}	Lag time (h)
Control gel	DS-A	0.698±0.7	0.078±0.10	10.20±0.39	3.20±0.49
DMSO	DS-B	0.894±1.09	0.093±0.12	23.33±0.11	1.40±0.49
OA	DS-C	0.757±0.918	0.085±0.91	13.61±0.72	2.40±0.49
Menthol	DS-D	1.28±1.20	0.012±0.12	27.22±0.49	1.10±0.49

*Data given as mean±SD (n=3), DS: Diclofenac sodium, DMSO: Dimethyl sulfoxide, OA: Oleic acid, SD: Standard deviation

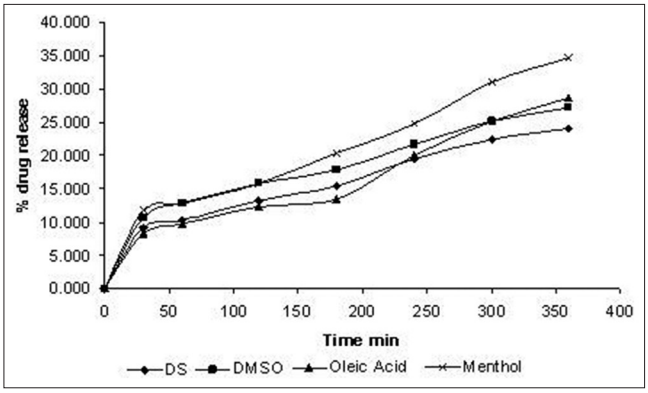

Fig. 3: Average % releases diclofenac sodium gel with a permeation enhancer through rat skin

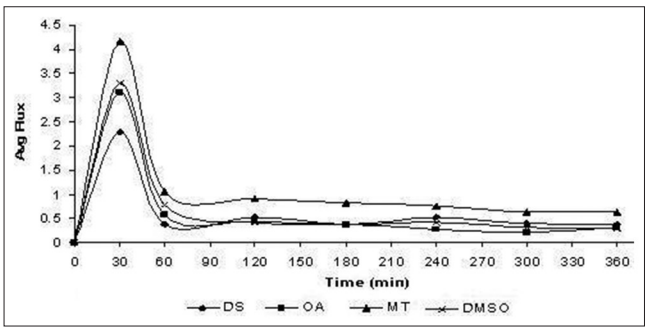

Fig. 4: Average flux of diclofenac sodium gel with permeation enhancer through rat skin

Gel containing DMSO showed a significant difference between the flux and kp of DS as compared to the control gel. DMSO is a dipolar aprotic solvent which is miscible with both water and organic solvents. It has the ability to accelerate the skin permeation of a wide variety of compounds including steroids, salicylates, and antimycotics. We preferred to test low concentration of DMSO, because we were concerned with the toxicity of DMSO at high concentrations [22]. Low concentrations of DMSO were reported to improve penetration for the drug piroxicam, prazosin, and methyl nicotine. However, we have seen some enhancing effect of DMSO 13% through cellophane membrane.

OA is an unsaturated fatty acid. It can interfere with the SC permeability barrier either (a) by forming pools of fluid within the SC or (b) by disrupting the molecular packing of the lipid matrix [12,23]. Results from this study indicate that the addition of 1% w/w OA 1% w/w significantly increased the flux, kp, and release rate of DS through rat skin compared to the control gel. The enhancement ratios (ER) (Table 3) were found to be 18% at the concentration of OA (1%, w/w).

Menthol is a trepen compound-containing alcohol that has been widely used as skin penetration enhancers for a variety of compounds. Menthol was selected for our studies, because it is also a refrigerant agent that induces a strong cooling sensation. When applied to the skin and numbs the sensation of pain, for this reason, it may provide an advantage for analgesic topical formulations [24,25].

Menthol at the concentration of 1% (w/w) has shown maximum permeation enhancement effect with an ER 40% (Table 6). The mechanism of action of terpenes has been intensively studied. Reported that permeation enhancement of menthol could involve its distribution into the intercellular space of SC and the possible reversible disruption of the intercellular lipid domain. This would increase the drug diffusivity [26-28].

Table 6: DS with permeation enhancer permeation parameter through rat skin

Enhancer	Gel code	Steady state flux (μg/cm²/h)	Kp (×10³) (cm/h)	Diffusion coefficient 10^{-8} cm×h^{-1}	Lag time (h)
Control gel	DS-A	0.87±0.85	0.08±0.12	9.60±0.43	3.40±0.49
DMSO 1%	DS-B	0.86±1.03	0.08±0.13	23.33±0.11	1.40±0.49
OA	DS-C	0.82±0.78	0.098±0.1	13.33±0.82	2.40±0.49
Menthol	DS-D	1.21±1.13	0.12±0.12	65.33±0.41	0.50±0.49

*Data given as mean±SD (n=3), DS: Diclofenac sodium, DMSO: Dimethyl sulfoxide, OA: Oleic acid, SD: Standard deviation

Table 7: ER of permeation enhancer incorporated in 1% (wt/wt) DS gel through cellophane membrane and rat skin

Enhancer	Gel code	Cellophane membrane %	Rat skin %
DMSO	DS-B	13	18
OA	DS-C	19	18
Menthol	DS-D	44	40

DS: Diclofenac sodium, DMSO: Dimethyl sulfoxide, OA: Oleic acid, ER: Enhancement ratio

CONCLUSION

The present investigation was carried out to explore the possibility to deliver therapeutically effective amounts of a gel formulation of DS. The addition of permeation enhancer to transdermal delivery systems may improve the penetration of drugs by modifying the thermodynamic activity of penetrants (e.g., changes in partitioning tendencies) or by altering the skin barrier properties (e.g., changes in fluidity of extracellular lipids). This study shows that a DS gel with carbopol with menthol 1% w/w can potentially deliver therapeutically relevant amounts of DS through skin.

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