Transdermal Delivery of Aceclofenac through Rat Skin from Various Formulations: A Comparative Study

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Summary

The aim of this study was to evaluate and compare the in vitro and in vivo transdermal potential of gel (G) and patch formulation (P) for aceclofenac (AC). The effect of different penetration enhancers were also examined when it was added to the formulations. To study the in vitro potential of these formulations, permeation studies were performed with Franz diffusion cells using excised dorsal rat skin. To investigate their in vivo performance, a carrageenan-induced rat paw edema model was used. The commercial formulation of aceclofenac (C) was used as a reference formulation. The in vitro permeation studies found that G was superior to P and C and that adding permeation enhancer to the formulations increased the permeation rate. The permeability coefficients (Kp) of AC from G and P were higher (Kp = $0.3465*10^{-2}$ cm/h and $0.228 *10^{-2}$ cm/h respectively than the Kp of AC from C = $0.1314 *10^{-2}$ cm/h. In the paw edema test, G showed the best permeation and effectiveness. The in vitro and in vivo studies showed that G could be a new, alternative dosage form for effective therapy.

Keywords: aceclofenac, in vitro permeation, carrageenan-induced rat paw edema test

Introduction

Transdermal drug delivery offers many important advantages. For instance, it is easy and painless, it protects the active compound from gastric enzymes, and it avoids the hepatic first-pass effect. Also, it is simple to terminate the therapy if any adverse or undesired effect occurs. But skin is a natural barrier, and only a few drugs can penetrate the skin easily and in sufficient quantities to be effective. Therefore, in recent years, numerous studies have been conducted in the area of penetration enhancement.[1,2] Penetration enhancers such as hydrogenated soybean phospholipids,[3] ethanol, alcohols with long carbon chains (C_8 to C_{14}), n-octanol and cyclic monoterpenes,[4,5] nonionic surfactants,[6] propylene glycol, and isopropyl myristate[4,5,7] have been used in many studies to increase the percutaneous absorption of drugs. Membranes from rats, mice, pigs, guinea pigs, snakes, rabbits, and humans as well as synthetic membranes have been used for these drug diffusion studies.

Although human cadaver skin may be the first choice as a skin model for a study of a final product to be used in humans, it is not always easy to obtain, and rat skin is a commonly used substitute.[8,9] Al-Saidan et al showed that in vitro permeation studies using rat skin could provide information useful for manipulating the design of transdermal therapeutic system (TTS) patches so that the desired permeation of the drug across human skin would be achieved.[10] Therefore, we used rat skin as a model membrane for our permeation studies. Gels are generally well known transdermal therapeutic systems. However, developing a gel and the subsequent penetration of the drug candidate in therapeutic amounts through the skin represent a significant challenge. This is especially true because gel should combine both pharmaceutical and cosmetic qualities. The hydrogel formulation was prepared and studied for its permeation potential and compared with the patch formulation.

AC is a nonsteroidal antirheumatic agent that has a potent anti-inflammatory effect, but it does not penetrate well through skin and cannot reach the effective concentration at the site of action after transdermal application.[4] For this reason, we wanted to suggest new, alternative dosage forms for transdermal application of AC. G and P formulations were developed and in vitro transdermal penetration of these formulations was compared with that of C. Furthermore, a pharmacodynamic study of AC was evaluated for its anti-inflammatory activity on a carrageenan-induced rat paw edema model for all formulations. This study aimed to both suggest a new, alternative dosage form for enhancing topical penetration of AC and to compare the prepared formulations with the commercial formulation available, evaluating the potential for penetration and transdermal absorption.

Materials and Methods

Materials

Eudragit RL 100 was kindly supplied by Zydus Cadilla, Ahmedabad India. Penetration enhancers, Triethanolamine and carrageenan were purchased from Sigma (St Louis, MO). AC was kindly provided by Ranbaxy Research Laboratories, Gurgoan India. All other chemicals used were of analytical grade.

Preparation of Topical Formulations

For the preparation of gels the specified quantity of polymers were taken and dispersed in the distilled water. After complete dispersion the polymers dispersion was kept in dark for 24 h to allow the complete swelling of the polymers. The specified quantity of ethanol was added in the aqueous dispersion of polymers. Then other ingredients like PEG -400, PG and triethanolamine (TEA) were added to get homogenous dispersion of gel. For the preparation of Patch formulation various placebo films were first casted onto the mercury surface for the selection of satisfactory films for drug incorporation. A film casting glass ring was fabricated for this purpose. Different proportions of polymers, plasticizers and penetration enhancers were accurately weighed and dissolved in a fixed volume of chloroform (5ml/10ml), using mechanical shaker (15 – 30 min.). The resulting solution was poured carefully into the glass ring kept on mercury surface in a petridish upon which an inverted funnel was placed to control the

evaporation rate of solvent. This minimized the chances of cracking or wrinkling of the films. The open end of the funnel was plugged with cotton to allow uniform evaporation of the solvents. The solvent was allowed to evaporate undisturbed. The films got dried up in approximately 24 h (Table-1).

Table -1. Contents of the and optimized Gel & Patch Formulation

Patch	**AC (mg) 50**	**Gel**	**AC (gm) 3.4%**
Eudragit RL100 (mg)	400	Eudragit RL 100 (gm)	1
PVP K-30 (mg)	100	Ethanol (%w/w)	25
Propylene Glycol (% w/w)	20	PEG-400 (%w/w)	15
PEG 400 (% w/w)	20	PG (%w/w)	15
Brig 58 (% w/w)	2	TEA (gm)	0.5
Chloroform (ml)	10		
Eudragit RL100 (mg)	400		
Distilled water (q.s.)			

Assay of Aceclofenac

A spectrophotometric analysis was used to determine AC permeation. First, 25 to 500 µL of stock solution (10 mg/10 mL, adjusted with phosphate buffer pH 7.4) was transferred by microsyringe into the 10 mL volumetric flasks. Then the volume was adjusted with phosphate buffer pH 7.4. The absorbances of the solutions were determined against a blank spectrophotometrically (LABOMED UV) at 275 nm. The aliquots of permeated formulations without AC were used as a blank. A calibration curve was then obtained in which Y was concentration (µg/mL), X was absorbance, and r^2 was 0.999. The sensitivity was 2.5 to 50 µg/mL. The limit of detection was 7.5 µg/mL.

Determination of Drug Solubility

To determine the drug solubility, an excess amount of AC was added to distilled water. This suspension was stirred at room temperature for 24 hours with a magnetic stirrer. The sample was then filtered through a 0.45-µm cellulose acetate filter. The concentration of AC was determined spectrophotometrically at 275 nm.[18,19]

Determination of n-Octanol–Distilled Water Partition Coefficient

n-Octanol phases were saturated with distilled water for at least 24 hours before the experiment. A solution of AC (10^{-4}M) was prepared with distilled water. Then, 2 mL of this solution was transferred to 10-mL assay tubes containing 2 mL of the organic phase. The tubes were stoppered and agitated for 24 hours at room temperature.

After centrifugation at 13500 rpm for 15 minutes, the concentration of the drug in the water phase was analyzed spectrophotometrically; the concentration of the drug in n-octanol was calculated from the difference between the initial and final concentrations in the water phase. Six replicates were used for the concentrations of n-octanol–distilled water solutions for partition coefficient calculations.[20]

In Vitro Permeation Studies

Vertical Franz-type diffusion cells (locally fabricated) with a diffusional surface area of 7.46 cm^2 were used to study the permeability of AC. The animal study protocol was reviewed and approved by the Ethics Committee at the Department of Pharmaceutical Sciences of Kashmir University. Skin samples were obtained from male Swiss albino rats weighing 140 to 160 g. After hair was shaven using a mechanical hair clipper, without damaging skin, a 5 × 5 cm patch of skin was excised from the dorsal region of each sacrificed rat. The excised rat skins were stored at –20°C. The skin membranes were first hydrated for 30 minutes in the buffer solution (pH 7.4) at room temperature (23°C) to remove extraneous debris and leachable enzymes. They were then placed between the donor and receptor compartments of the cells, with the dermal side in direct contact with the receptor medium. Approximately 20 mL of phosphate buffer (pH 7.4) was placed in the receptor compartment. Its temperature was maintained at 37 ± 0.5°C using a thermostatic water bath and it was stirred at 100 rpm throughout the experiment. The donor compartment contained 0.5 g of gel and 8.80cm^2 of the patch. The aliquots (1ml) were withdrawn at predetermined time intervals and then immediately analyzed spectrophotometrically at 275 nm against a blank prepared with the permeated formulation without the drug. The same amount of fresh buffer was added to the receptor compartment to replace what had been removed. Three replicates of each experiment were performed.

Anti-inflammatory Test

The rats were divided into four groups (n=6) and were given free access to water and food. The rats were kept under observation for 24 h. The abdominal side of rats were shaved 12 h before starting the experiment. The transdermal Gel, patch and marketed gel were applied on the shaved backs of all animals of group no. 1, 2 and 3 respectively (fourth groups served as control with no treatment). Right hind paw edema was induced in all four groups of animals by subplanter injection of 0.1 ml of a 1% w/v homogeneous suspension of carrageenan in distilled water. In animals of group no. 1, 2 and 3 carrageenan was injected half an hour later the application of formulations. The swelling of the injected paw was measured immediately (0 h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h after injection using a digital plethysmometer. The amount of paw swelling was determined from time to time and expressed as percent edema relative to the initial hind paw volume.

Results and Discussion

After addition of AC to G or P, no opalescence was observed, indicating that these systems retained their stability when the drug was added.

The solubility of AC in distilled water in this study was found to be 0.086 mg/mL. The partition coefficient of AC in n-octanol–distilled water was calculated as 5.27.

For the gel formulation, 0.5 gm of G in the donor compartment contains 17 mg AC. For the patch formulation, 8.80 cm^2 contained 50 mg of the drug.

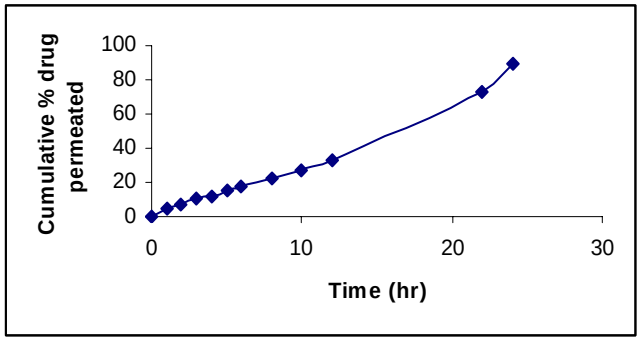

Fig: 1 *In-vitro* skin permeation studies of optimized gel formulation

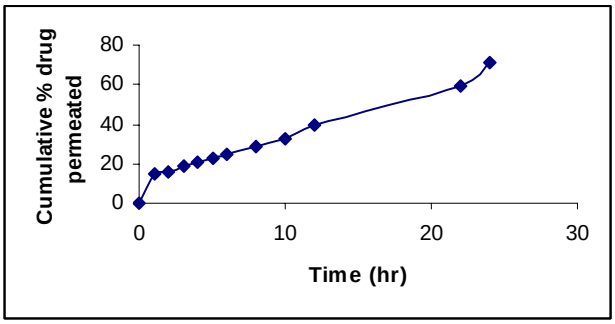

Fig: 2 *In vitro* permeation pattern of optimized patch formulation

Steady-state flux (J) and the Permeability coefficient (Kp) were calculated from the slope of the linear portion of the graph.[25, 26]

The optimized formulations of patch and gel were further evaluated for *in-vitro* and *iv-vivo* characteristics for comparison. The comparative result of *in vitro* skin permeation (Fig 1-2) and *in vivo* (anti-inflammatory) (Fig. 3-5; Table 2-5) studies of optimized formulation of gel and patch is given below.

Table-2: % Edema data w.r.t. initial paw volume obtained in Wistar rats (Control group)

Rat No.	Initial Paw Vol. (ml)	Time (h)									
		0.5	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**24**
1	1.17	6.83	12.82	10.25	56.41	76.92	102.56	111.96	129.91	159.82	172.64
2	0.89	6.02	5.61	16.85	20.22	30.33	40.44	52.80	61.79	67.41	74.15
3	0.91	5.49	15.38	29.67	37.36	41.75	52.74	74.72	96.70	106.59	129.67
4	0.81	4.93	17.28	16.04	24.69	29.62	37.03	41.97	46.91	48.14	59.25
5	0.70	8.57	12.85	21.42	27.14	41.42	44.28	50.00	51.42	55.71	64.28
6	0.98	13.26	15.30	18.36	21.42	27.55	33.67	37.75	46.93	57.14	69.38
Mean % Edema ± SD n = 6		7.51 ±3.91	13.20 ±3.2	18.76 ±4.5	31.20 ±9.32	41.26 ±14.15	51.78 ±20.23	61.53 ±20.6	72.27 ±28.25	82.46 ±32.15	94.89 ±39.05

Table-3: % Edema data w.r.t. initial paw volume obtained in Wistar rats (Eudragit gel)

Rat No.	Initial Paw Vol. (ml)	Time (h)									
		0.5	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**24**
1	0.98	0	0	0	0	0	0	0	0	0.00	0.00
2	0.82	0	0	0	0	0	0	0	0	8.54	8.54
3	1.04	0	0	0	0	0	0	0	0	5.77	4.81
4	0.97	0	0	0	0	0	0	0	0	4.12	12.37
5	0.71	0	0	0	0	0	0	0	0	5.63	14.08
6	1.02	0	0	0	0	0	0	0	0	0.98	9.80
Mean % Edema ± SD n = 6		0	0	0	0	0	0	0	0	4.17 ±3.20	8.27 ±5.17
% Inhibition w.r.t. Control group		100	100	100	100	100	100	100	100	95.26	91.71

Tabl-4: % edema data w.r.t. initial paw volume obtained in Wistar rats (Optimized Patch)

Rat No.	Initial Paw Vol. (ml)	Time (h)									
		0.5	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**24**
1	0.89	1.12	1.12	1.12	0	0	0	1.12	2.24	14.60	19.10
2	0.96	0	0	0	2.08	2.08	1.04	2.08	7.29	12.50	12.50
3	0.96	1.04	1.04	1.04	1.04	1.04	2.08	0	1.04	1.04	1.04
4	0.95	0	0	0	0	1.05	1.05	1.05	2.10	13.68	23.15
5	0.91	0	0	0	0	0	2.19	2.19	2.19	1.09	0.00
6	0.90	0	1.1	0	2.2	0	1.1	0	6.66	13.33	16.66
Mean % Edema ± SD n = 6		0.36 ±2.89	0.54 ±3.87	0.36 ±2.93	0.88 ±2.91	0.69 ±3.87	1.24 ±4.95	1.07 ±4.89	3.58 ±1.88	9.37 ±3.86	12.07 ±2.54
% Inhibition w.r.t. Control group		95.20	95.90	98.84	97.86	98.65	97.98	98.26	95.04	88.63	87.28

Tabl-5 : % edema data w.r.t. initial paw volume obtained in Wistar rats (Marketed Gel)

Rat No.	Initial Paw Vol. (ml)	Time (h)									
		0.5	**1**	**2**	**3**	**4**	**5**	**6**	**8**	**12**	**24**
1	1.05	0	0	0	0	3.80	25.71	32.38	24.76	29.52	30.47
2	1.02	0	0	0	16.66	16.66	19.60	36.27	32.35	36.27	38.23
3	0.89	0	0	0	3.37	13.48	22.47	22.47	25.84	14.60	16.85
4	0.75	0	0	1.33	1.33	4.00	5.33	17.33	18.66	10.66	81.33
5	1.04	0	0	0	5.76	4.80	25.96	27.88	29.80	28.84	30.76
6	1.03	0	0	1.94	3.88	15.53	17.47	20.38	33.00	33.00	33.98
Mean % Edema ± SD n = 6		0 ±3.79	0 ±3.81	0.545 ±3.75	5.166667 ±4.12	9.711667 ±3.69	19.42333 ±4.32	26.11833 ±5.01	27.40167 ±2.88	25.48167 ±3.66	38.60333 ±3.78
% Inhibition w.r.t. Control group		100	100	97.09	83.46	76.49	62.49	57.56	62.08	69.10	59.32

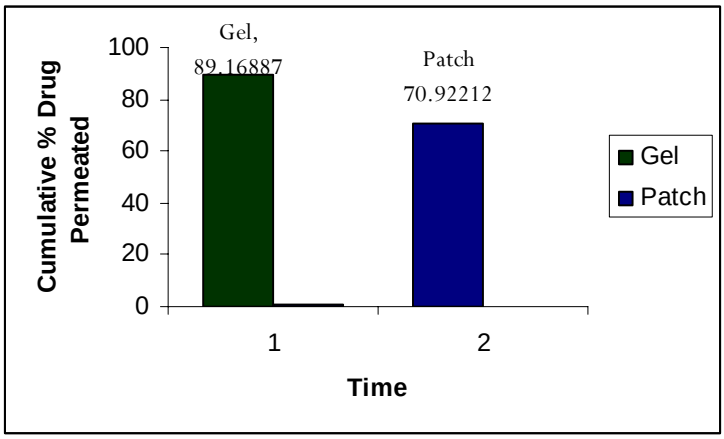

Fig: 3 Comparison of cumulative % permeated by gel and patch formulations

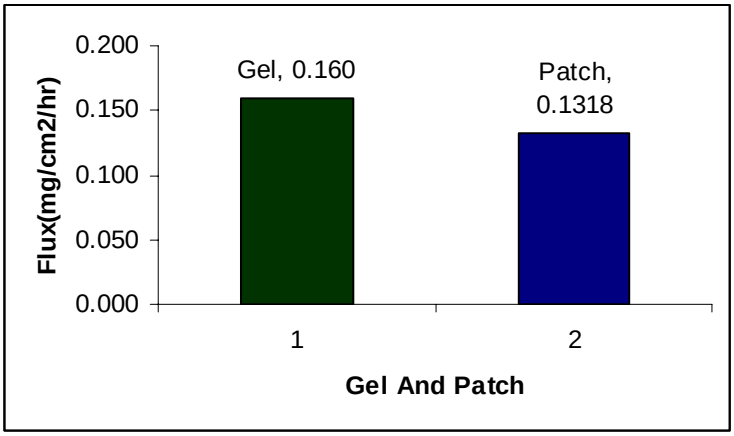

Fig: 4 Comparison of flux (permeation rate) between the gel and patch

Better permeation of aceclofenac was obtained by gel as compared to Patch through transdermal route.

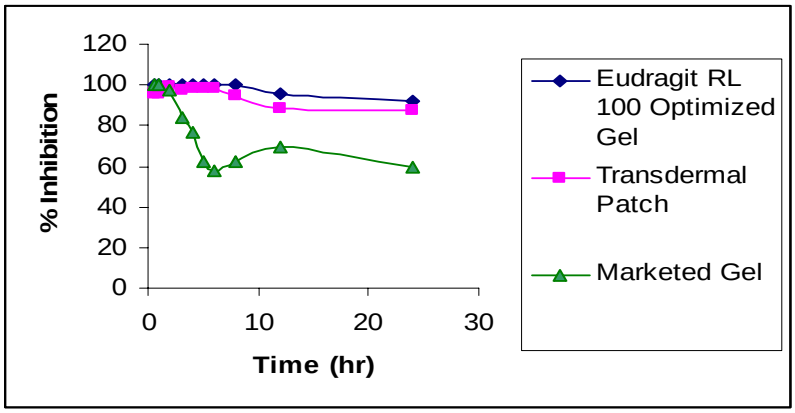

Fig: 5 Comparison of percent inhibition of edema between optimized Eudragit gel, patch and marketed gel.

Gel exhibited 100% inhibition of edema for longer period of time (upto 8 h) when compared to patch (98%upto 4 h).

It was seen that optimized formulation of gel & patch showed greater *in vitro* drug release and permeation respectively. In addition, the permeability coefficients were found to be higher for these formulations. This could be attributed due to the presence optimum concentration of permeation enhancer in each formulation. The highest release shown by Eudragit RL100 can be attributed due to the free permeability to water leading to the leaching of the soluble component, which leads to the formation of pores and thus a decrease in the mean diffusion path length of drug molecules to release into the dissolution medium. The result is higher dissolution rates and eventually higher permeation. Besides this, Eudragit RL 100 has 10% of functional quarternary ammonium groups. The ammonium groups are present as salts and give rise to pH independent permeability of the polymers, give rise to lesser fluctuation in the release and permeation and hence sustained effect.
The highest flux achieved by nonionic surfactant, Brij 58 may be due to its large hydrophobic content (C_{16} in case of Brij 58) and to much greater oxyethylene content in Brij 58 (20 OE units) indicating that the solubilization site of drug between the hydrophilic head groups of polyoxyethylene (POE) groups leading to the enhanced flux and permeability coefficient. Apart from this, increase in the flux can also be ascertained due to the hydrogen bonding between the –OH and –NH group of surfactant and the Aceclofenac molecules respectively.
The anti-inflammatory activities of optimized formulations of Optimized Gel & Patch were evaluated using the carrageenan induced hind paw edema method.
Mean percent edema of all groups was calculated. Percent inhibition of edema was determined with respect to control group in those groups in which formulations were applied. Inhibition of edema was found to be highest in the groups in which Eudragit RL 100 gel was applied. The gel inhibited the edema 100 % upto 8 h, and then it was 95.26 % after 12 h and 91.71 % after 24 h. Transdermal patch inhibited the edema 95.20 % upto 0.5h and then it was 95.90%, 98.84%, 97.86, 98.65, 97.98, 98.26, 95.04, 88.63 and 87.28% inhibition after 1h, 2 h, 3h, 4h, 5h, 6h, 8 h, 12 h and 24 h respectively. Aceclofenac marketed gel formulation inhibited the edema 100% only upto 2 h and then it was 59.32 after 24 h.
Based on the anti-inflammatory studies it can be concluded that Aceclofenac Eudragit gel formulation gives maximum inhibition of edema than the patch, which in turn is far better than marketed gel formulation.
Comparative views of cumulative percent drug permeated, flux and percent inhibition of edema w.r.t control group was presented between Gel and Patch of Aceclofenac. Cumulative percent drug permeated (89.168 %), flux (0.160 mg/cm^2/h) and % inhibition (100 % inhibition of edema upto 8 h) of Gel was found to be higher than patch (Cumulative percent permeated -70.92 %, flux (0.1318 mg/cm^2/h) and percent inhibition – 100 % upto 4 h) .

From the above studies, it can be inferred that the Eudragit gel is a better delivery system than patch through transdermal route.

Conclusion

This study demonstrated that formulating aceclofenac into Gel shows enhanced drug penetration through rat skin in vitro and in vivo. Gel containing aceclofenac may offer promise as an anti-inflammatory dosage form, ensuring more effective therapy, but additional extradermal tests and experiments should be performed before the formulation is used in humans.

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