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Research Article

EFFECT OF IONTOPHORESIS AND PENETRATION ENHANCERS ON THE IN VITRO DIFFUSION OF A PIROXICAM GEL

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ABSTRACT

The stratum corneum behaves as a barrier for most of drugs percutaneous absorption into the body. The ability of drug to penetrate the stratum corneum can be improved by using physical and chemical methods. The purpose of this study is to determine the effect of penetrance enhancers addition and application of iontophoresis method on diffusion of piroxicam gel preparation in vitro. The study was performed with flow through method for 6 hours using porcine ears skin as a diffusion membran; ethanol, ethyl acetate, tween 80 and dimethyl sulfoxide (DMSO) as penetrance enhancers; and 0.5 mA/cm^2 constant current for iontophoresis method. The result showed that the piroxicam diffusions from the formula with penetrance enhancer were : F1 (ethanol 5%) > F3 (ethyl acetate 5%) > F4 (Tween 80 5%) > F2 (DMSO 5%) > FS (without penetrance enhancer) respectively. The amount of drug diffused by iontophoresis method was twice higher than in original formula (FS) without iontophoresis. The combination effect of penetrance enhancer and iontophoresis showed a better synergic effect in the formula F1 and F3, while in F2 and F4 was unclear.

Keywords: Iontophoresis, Piroxicam, Penetration enhancer, Difussion, ethanol, Dimehtylsulfoxide, Ethyl acetate, Tween 80

INTRODUCTION

Transdermal drug delivery route provides severaladvantages[123] :

- It avoids GIT side effect, inactivation of drug by GIT enzymes, interaction of drug with food and first-pass metabolism of drugs in GIT.
- It provides controlled and sustained release of the medicament.
- It improves the bioavailability of drug.
- It provides uniform drug plasma concentration.
- It improves the patient's compliance.
- It can be administered to non- responsive, unconscious and nauseating patient.
- It provides easy termination of drug in case of toxicity by removal of the formulation from the skin.

However, only few drug molecules have been formulated into transdermal patches because of the low permeability of the skin. The outermost layer of skin, the stratum corneum, forms a barrier against permeation of drugs into the body. Increasingpermeability of stratum corneumcan be done with penetration enhancers (chemical method) and iontophoresis (physical methods)[4].

Penetrationenhancers is a compound that can reduce the ability of the stratum corneum barrier by reacting with components of the stratum corneum(i.e lipid, protein orceratin). iontophoresis, which is the facilitated movement of ions across a membrane under the influence of

an externally applied small electrical potential difference (0.5 mA/cm2 or less), is one of the most promising novel drug delivery system, which has proved to enhance the skin penetration and the release rate of a number of drugs having poor absorption/permeation profile through the skin[5].

Piroxicam, one of the non-steroid anti-inflammatory drug (NSAID), is used as a model in this study and has two pKa values (1.8 and 5.2) depending on the group pyridil and enol. Depending upon pH, therefore, the drug can exist in cationic, neutral (i.e.,zwitterionic) or anionic forms. The passive transport of piroxicam across mammalian skin is relatively low[6].

In the in vitrostudy of piroxicam gel diffusion through the impregnated membranewith a solution of Spangler, diffusion of piroxicamgel formulation with the solvent alcohol was better than the water solvent[7]. In another study, the permeation rate of piroxicam gel with the addition of dimethyl sulfoxide (DMSO)penetration enhancer was almost five times faster than without it[8]. Iontophoresis transdermal delivery of piroxicam gel in combination with oleic acid showed the synergistic effect in increasing passive diffusion through biological membranes [9].

The purpose of this study is to determine the effect of penetrance enhancers addition and application of iontophoresis method on diffusion of piroxicam gel preparation in vitro.

MATERIALS

Piroxicam (PT.Indofarma, Tbk), Carbopol 940, DMSO, methanol (GT Baker), citric acid monohydrate, phosphate buffer (Na2HPO4.12 H20, NaHPO4.1H2O), HCl, NaOH, Triethanolamine, KCl, ethyl acetate, ethanol 95%, Tween 80 (PT. Bratachem).

METHODS

Preparation of Piroxicam Gel

The piroxicam gels were Carbopol-based (Carbopol 940). Carbopol powders were allowed to swell in the water base for 24 hours and then were mixed using VirtiShear kinetic mixer until a homogeneous base gel was obtained. Piroxicam was dissolved in a mixture of 10 ml distilled water and 3 ml Triethanolamine. Base gel and enhancers was inserted into the piroxicam solution and thendistilled water was added until it reaches 100 ml, stirred until a homogeneous gel was formed.

Evaluation of Piroxicam Gels

All of formulas would be evaluated by examination of organoleptic, measurement of viscosity using a Brookfield Viscometer DV-II, and pH value of the preparation using a Beckman pH meter, homogenity of piroxicam with HPLC (Waters)method.The optimum conditions obtained in the combination of methanol: buffer (70:30, v / v). Buffer used was 7.72 g of anhydrous acetic acid and 5.35 g of sodium phosphate which was turned into base in 1000 ml and the pH were adjusted to 4 by the addition of 1N NaOH. The stationary phase used a SGE C 18 column (250x4.6 mm C18 RS SS Wakosil 5µm) with a flow of 1 mL / min.

Samples were prepared by weighing 0.1 g of gel which was equivalent to 50 mg/mL piroxicam and was dissolved in 0.01N hydrochloride methanol until 10 mL. Samples solution were filtered with Millipore filters (0.42 µm) and injected into HPLC.

Table 1: Formulation of Gel piroxicam

Material	F_s	F_1	F_2	F_3	F_4
Carbopol 940	1	1	1	1	1
Piroxicam	0,5	0,5	0,5	0,5	0,5
Trietanolamin	3	3	3	3	3
Etanol	-	5	-	-	-
Dimethyl sulfoxide	-	-	5	-	-
Ethyl acetate	-	-	-	5	-
Tween 80	-	-	-	-	5
Distilled water ad	100	100	100	100	100

Preparation of Membrane

Porcine ears were obtainedfrom a slaughterhouse. The skin was carefullyremoved leaving the fat tissue behind. Any skin,in which the barrier was disrupted, was removed.The skin was cut into 2 cm x 2 cm samples for permeation studies[10]. Thickness of skin tissue is 1200 µm (full thickness). Skin immediately stored at -20 º C until the experiments were carried out[11].

Preparation of Electrodes

Iontophoresis experiments were conducted using silver/silver chloride electrodes. The silver chloride electrodes were prepared as follows: silver wires (0.1 cm diameter; length 3.1 cm) were immersed in 0.1N HCl solution and connected to the anode of an amperostaticstate (1 mA) and time of electrolysis 6 hours with 0.1 M KCl.

Preparationof Iontophoresis

The Iontophoresis tools were newly-designed in this study, collaboration with Biomedical Engineering Laboratory, School of Electrical Engineering and Informatics, InstitutTeknologi Bandung. The series were set to produce a constant current density of 0.5 mA/m2. The amperemeter was used for calibrating constant current before the experiment was carried out.

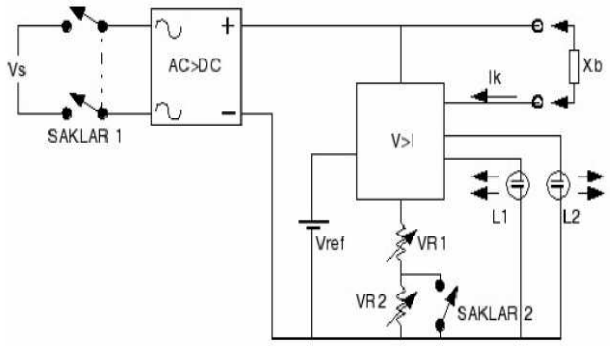

Fig. 1: Diagram of Constant Current Power Supply Powerful Iontophoresis

***In vitro* diffusion test[8]**

In vitro diffusion test was performed using the flow through methodwith Modified-Franz diffusion cell (Figure 2). All formulaswas weighed as much as 1.0 g, flattened above the membrane with a surface area of 2 cm^2. System's temperature 37 ± 0.5 ºC with the receptor phosphate buffer pH 7.4 (2.77 g Na_2HPO_4. $12H_2O$ and 0.31g Na_2HPO_4. $12H_2O$in 200ml) about 50 mL. Each process were carried out for 6 hours without and with iontophoresis. Aliquotwere taken from the receptor fluid as much as 5 ml and replaced with phosphate buffer pH 7.4 at the 30^{th}, 60^{th}, 120^{th}, 180^{th}, 240^{th}, and at the 360^{th} minutes, then were analyzed by HPLC method .

RESULTS AND DISCUSSION

Piroxicam gel formulas with Carbopol 940asthe base had several advantages, such as : it can easily dispersed in water due to hydrophilic groupand in small concentrations (0.02-2%) can be used as base gel with sufficient consistency and it is easy to use and wash with water. Using Carbopol 940 showed good result in organoleptic test in each formulas. All formulas showed pH in range 7.8 to 8.2 which indicatedpiroxicam were in anionic form and hada negative charge, Therefore, the movement flow of the ion with iontophoresis techniques were from the cathode to the anode. Viscosity testing showed that FS wasthe highest (1378.00cp) and F4 the lowest (1247.33 cp) viscosity (table 2).

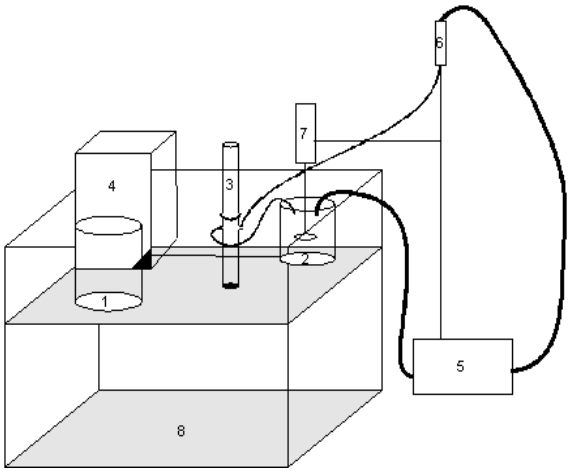

Fig. 2: *Invitro* Diffusion Test[8]

1 = Receptor fluid replacement, 2 = receptor compartment , 3 = donor compartment ,4 = thermostat, 5 = peristaltic pump, 6 = busting bubbles, 7 = stirrer, 8 = water bath

Penetrationenhancers substances, 5% in each formulas except FS, decreasing the consistency of the gels because of the incorporation with the Carbopol 940. Adding Triethanolaminein the formulas maked Carbopol 940 more viscous by changed to alkaline condition and triethanolam in which has a pH value of 10.5 increasing the solubility of piroxicam which has very little soluble in water and organic solvents but soluble in alkaline pH. Piroxicam content uniformity test showed all formulas are relatively homogeneous.

In vitro diffusion without iontophoresis study showed F1, Ethanol 5% as penetration enhancer, was the higest diffusion rate (Figure 3).The solubility of piroxicam with the addition of ethanol was increasing. Besides that, ethanol has ability to extract the lipids resulting in changes in the thickness of lipid layer in the stratum corneum. The highest rate of diffusion were F1 > F3 > F4 > F2 > FS respectively.

The diffusion rate of piroxicam gel with iontophoresis applicationand absence the penetration enhancer (FS) was almost two times faster than without it (Figure 4). Permeation pathway of drug molecules with iontophoresis method is through the pore (transgranular and transfollicular) and intercellular (corneocytes gap)[12]. The existence of an electric current 0.5 mA/cm^2 cause a reaction on the electrodes that generate the flow of electrons and encourage the emergence of piroxicamelectromigration of ions through the pores of the stratum corneum and corneocytes gap. The process of ion transport through the skin occurs as a process to maintain a neutral electrical charge (electro-neutrality).

Table 2: Result of piroxicam gel preparation

Test	Test Formula				
	FS	**F1**	**F2**	**F3**	**F4**
Organoleptic					
- Color	-Yellow transparent	-Yellow transparent	-Yellow transparen	-Yellow transparent	-Yellow transparent
- Smell	- No smell	- No smell	- No smell	- No smell	- No smell
pH	8,11±0,01	8,13±0,01	8.24±0,01	7.80±0,01	8.20±0,01
Viscosity (P)	1378,00±48,04	1260,00±34,64	1324,00±3,60	1247,33±45,34	1261,33±8,14
The content of piroxicam* (µg/mL)	50,66±2,10	49,62±0,40	49,99±1,56	49,48±0,50	50,18±1,22

FS: Formula without penetration-enhancing substances; F1: Formula with 5% ethanol; F2: Formula with 5% DMSO

F3: Formula with 5% ethyl acetate; F4: Formula with 5% Tween 80; *In every 0.1g of gel

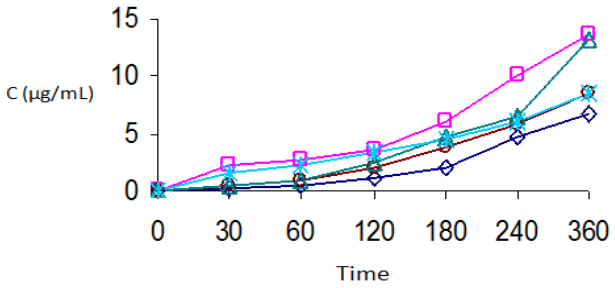

Fig. 3: Curve of the rate of diffusion of piroxicam gel without iontophoresis..-◊- FS(Without penetrationenhancers substances). -□- F (ethanol), -○-F2 (DMSO), -Δ-F3 (Ethyl acetate), -*- F4 (Tween 80)

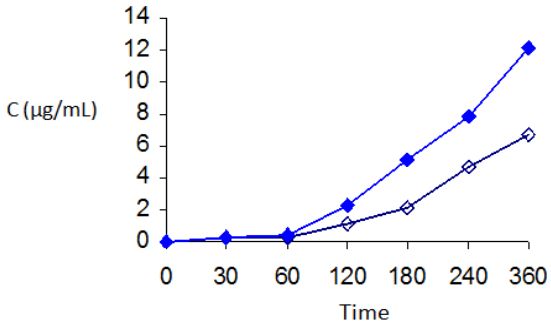

Fig. 4: Curve of the rate of diffusion of piroxicam gel with and without iontophoresis, -♦-FS (With iontophoresis), -◊- FS (Without iontophoresis)

The effect of combination of chemical (penetrationenhancers substances) and physics (iontophoresis) methods in the rate of diffusion can be seen in the figures 5 and 6. The use of a combination of techniques and substances iontophoresis penetration enhancers provided a synergistic effect on F1 and F3 formulas which gavethe result higher than the diffusion of piroxicam by penetration enhancers substances or methods iontophoresis separately. This could happen because there is no mutual interaction inhibits the increase in the rate of permeation mechanism of each method. Mechanism of penetration enhancement by ethanol because of the increased solubility of piroxicam whereas ethyl acetate through increased lipid fluidity and porosity of the stratum corneum, making it easier for ions to diffuse piroxicam with electromigration mechanism. In previous studies, Srinivasan*et al* proved that the influence of ethanol and transdermal delivery of leuprolideiontophoresis at the rate of diffusion can increase by several times[13].

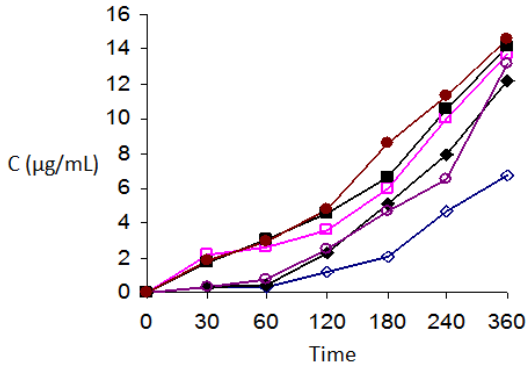

Fig. 5: Curve of the rate of diffusion of piroxicam gelFS,F1 and F3.

-◊- FS (without iontophoresis), -♦- FS (withiontophoresis),- ♦- F1 (without iontophoresis), -■-F1 (withiontophoresis),-○- F3 (without iontophoresis),- ●- F3 (with iontophoresis).

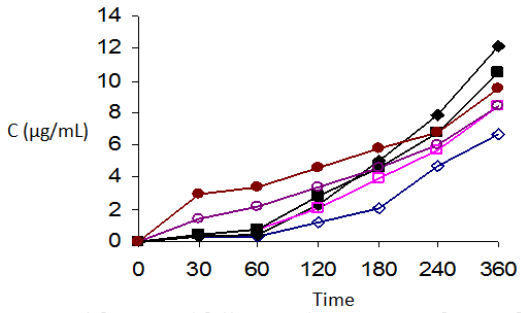

Fig. 6: Curve of the rate of diffusion of piroxicam gel FS,F2,dan F4.

-◊- FS (without iontophoresis), -♦- FS (with iontophoresis),-□- F2 (without iontophoresis),

-■-F2 (with iontophoresis), -○- F4 (without iontophoresis), - ●- F4 (with iontophoresis)

The use of the formula iontophoresis F2 and F4 had no synergistic effect in first 2 hoursal though there was an increase in the rate of diffusion in F4 in the next 4 hours, the iontophoresis method and the penetration enhancers substance as its own have a better rate of diffusion. The use of Tween 80 in the first 2 hours may change the permeability of the stratum corneum due to the association of groups with hydrophilic and hydrophobic structures *Brick and Mortar* stratum corneum. As a result, in terms of changes in the pores of oil and sweat glands will be widened, resulting in better diffusion when combined with iontophoresis, but Tween 80 which is a nonionic surfactant (neutral), also undergo the process of electro-osmosis through the skin, until the amount of Tween 80 in the F4 gel decreases with an influence of the pore stratum corneum. For formula F2, DMSO incompatibility of the oxidizing agent, presence of Ag with DMSO can reduces the effectiveness of penetration enhancers when combined with the method iontophoresis. This result agrees with the results of a previous study by Wearley *et al* that stated that the use of DMSO is no better combination than separately iontophoresis.

CONCLUSION

The result showed that the piroxicam diffusions from the formula with penetrance enhancer were : F1 (ethanol 5%) > F3 (ethyl acetate 5%) > F4 (Tween 80 5%) > F2 (DMSO 5%) > FS (without penetrance enhancer) respectively. The amount of drug diffused by iontophoresis method was twice higher than in original formula (FS) without iontophoresis. The combination effect of penetrance enhancer and iontophoresis showed a better synergic effect in the formula F1 and F3, while in F2 and F4 was unclear.

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