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Optimization of ibuprofen gel formulations using experimental design technique for enhanced transdermal penetration

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ABSTRACT

The aims of this study were to develop a transdermal gel formulation for ibuprofen using experimental design techniques and to evaluate its pharmacokinetic properties. The three factors chosen for factorial design were the concentrations of drug, polyoxyethylene(5)cetyl/oleyl ether and ethanol and the levels of each factor were low, medium and high. Skin permeation rates and lag times of ibuprofen were evaluated using the Franz-type diffusion cell in order to optimize the gel formulation. The permeation rate of ibuprofen significantly increased in proportion to the drug concentration, but significantly decreased in proportion to POE(5)cetyl/oleyl ether concentration. Ethanol concentration was inversely proportional to the lag time. The pharmacokinetic properties of the optimized formulation were compared with those of two marketed products in rats. The relative bioavailability of ibuprofen gel compared to the two marketed products was 228.8% and 181.0%. In conclusion, a transdermal ibuprofen gel was formulated successfully using the technique of experimental design and these results helped in finding the optimum formulation for transdermal drug release.

1. Introduction

Ibuprofen is a potent non-steroidal anti-inflammatory (NSAID) drug often used for the treatment of acute and chronic arthritic conditions (Busson, 1986). Like other drugs in this category, ibuprofen can cause gastric mucosal damage which may result in ulceration and/or bleeding. Therefore, there is a great interest to develop the topical dosage forms of ibuprofen to avoid the oral side effects and to provide relatively consistent drug levels at the application site for prolonged periods (Babar et al., 1990; Muktadir et al., 1986; Ranman et al., 1990). Topical drug delivery of ibuprofen in various formulations has been described in the literature (Muktadir et al., 1986; Chang et al., 1997; Brown et al., 2001; Iervolino et al., 2001; Takahashi et al., 2002; Lopez et al., 2004; Cilurzo et al., 2005; Swart et al., 2005; Chen et al., 2006). An effective permeation of ibuprofen, however, is difficult to achieve by transdermal delivery due to its intrinsically poor skin permeability, even though this is relatively good compared to other NSAIDs in common use (Chang et al., 1997).

Our previous study showed that polyoxyethylene(POE)(5)cetyl/oleyl ether was an effective non-ionic surfactant for the enhancement of the skin permeation of ibuprofen (Park et al., 2000). POE alkyl ethers are generally regarded as nontoxic and nonirritant materials (Gupta and Singh, 2005). Ethanol is widely used as a skin permeation enhancer at concentrations of up to 70% in many transdermal therapeutic systems (Krishnaiah et al., 2002; Al-Saidan et al., 2004; Fang et al., 2008). In addition, Krishnaiah et al. (2005) reported that a transdermal gel containing 70% ethanol showed no signs of skin irritancy when applied for 1 day in humans.

Designing drug delivery formulations with the minimum number of trials is very crucial for pharmaceutical scientists (Hamed and Sakr, 2001). In this study, we demonstrate the use of factorial designs to optimize the topical formulation for ibuprofen. Compared with one-factor-at-a-time experiments, a factorial experiment is more efficient in multi-factor optimization. More importantly, although the one-factor-at-a-time experiments can easily miss the optima, the factorial designs will give a combination near the maximum (Li et al., 2005). The objectives of this study were to develop a novel topical gel formulation for ibuprofen using POE(5)cetyl/oleyl ether and ethanol as skin permeation enhancers, and to evaluate its pharmacokinetic properties in comparison with cream type formulations containing 5% ibuprofen which are currently marketed. The present study was carried out to evaluate the effect of three factors (amount of ibuprofen, POE(5)cetyl/oleyl ether and ethanol) on drug skin permeation from gels and to optimize the level of these factors using response surface methodology in order to obtain the targeted skin permeation profiles (high flux and short lag time) for ibuprofen.

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2. Materials and methods

2.1. Materials

The following materials were used as received, without further purification. Ibuprofen was purchased from Sigma (St. Louis, MO, U.S.A.). Oxaprozin was obtained from Cilag AG Co. (Schaffhausen, Switzerland). Carbopol® 940 (BF Goodrich Co., U.S.A.), POE(5)cetyl/oleyl ether, polyethylene glycol(7) glyceryl cocoate (Henkel Co., Germany), heparin sodium (Choongwae Pharma Co., Korea), Senterlan® cream (Unicorn Laboratories, Hong Kong) and Ibutop® cream (Deutsche Chefaro Pharma GmbH, Germany) were used. Phosphoric acid (Shinyo Pure Chemical Co., Japan), monosodium phosphate, diethyl ether and triethanolamine (Junsei Chemical Co., Japan) were of analytical grade. All organic solvents were of high-performance liquid chromatography (HPLC) grade. All other chemicals were of reagent grade. Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, U.S.A.).

2.2. Preparation of drug formulation using the factorial designs

Experimental conditions for the factorial design parameters are shown in Table 1. Gel dosage forms of ibuprofen were prepared using a serial mixture of deionized water and ethanol as the vehicle and a gelling agent of Carbopol® 940 at a concentration of 1.5% (w/w). After complete hydration of Carbopol® 940 by the vehicle, drug, POE(5)cetyl/oleyl ether, PEG-7 glyceryl cocoate and triethanolamine were added and mixed completely, and then vehicle was added to give a total weight of 100 g. A 3^3 factorial design was constructed for the ibuprofen gel formulation (Table 2).

Table 1
Experimental conditions for the factorial design parameters

Factors	Levels[a]		
	Low (−)	Medium (0)	High (+)
Ibuprofen	2	5	10
POE(5)cetyl/oleyl ether	10	20	30
Ethanol	10	20	30
Carbopol® 940	1.5	1.5	1.5
PEG-7 glyceryl cocoate	15	15	15
Triethanolamine	0.5	0.5	0.5
Water	q.s. to 100 g	q.s. to 100 g	q.s. to 100 g

[a] Unit: g/100 g.

2.3. Optimization of ibuprofen gel formulation

A computer optimization technique was applied to optimize the design of the ibuprofen gel formulation, which contained POE(5)cetyl/oleyl ether and ethanol as absorption enhancers. The 3^3 factorial design and response surface design were applied to deal with response variables (skin permeation rates (J_s) and lag times (T_L)), which are given as quantitative data. The statistical analysis was performed using MINITAB 14 software (Minitab Inc., PA, U.S.A.) with general optimization techniques. The selected optimized ibuprofen gel formulation was prepared for the pharmacokinetic study in rats. The optimized formulation is described in Table 3. Each experiment was repeated six times and their mean value with standard deviation is presented.

2.4. Skin permeation of ibuprofen through excised hairless mouse skins

All animal studies were performed in accordance with the Principles for Biomedical Research Involving Animals developed by the

Table 2
Matrix of a 3^3 factorial design for the formulation of ibuprofen gel and permeation parameters of ibuprofen through hairless mouse skins from ibuprofen vehicles

Trial no.	Levels[a]			Permeation parameters[b]	
	Ibuprofen	POE(5)cetyl/oleyl ether	Ethanol	T_L (h)	J_s (μg/(cm^2 h))
1	−	−	−	3.24 ± 0.60[c]	47.33 ± 6.58
2	−	−	0	1.78 ± 0.54	48.18 ± 3.37
3	−	−	+	1.01 ± 0.39	54.65 ± 3.63
4	−	0	−	2.82 ± 0.40	42.26 ± 2.60
5	−	0	0	1.80 ± 0.22	35.93 ± 6.00
6	−	0	+	1.27 ± 0.20	29.14 ± 1.50
7	−	+	−	2.22 ± 0.37	17.74 ± 1.45
8	−	+	0	1.40 ± 0.30	53.70 ± 5.68
9	−	+	+	1.88 ± 0.28	32.45 ± 1.53
10	0	−	−	1.32 ± 0.45	125.36 ± 8.46
11	0	−	0	2.28 ± 0.13	153.21 ± 5.68
12	0	−	+	1.15 ± 0.14	164.45 ± 16.12
13	0	0	−	2.38 ± 0.73	112.44 ± 20.24
14	0	0	0	2.18 ± 0.12	126.24 ± 4.83
15	0	0	+	0.65 ± 0.14	53.87 ± 7.18
16	0	+	−	1.63 ± 0.56	75.88 ± 6.83
17	0	+	0	1.72 ± 0.12	78.40 ± 12.69
18	0	+	+	1.48 ± 0.15	68.38 ± 7.41
19	+	−	−	2.08 ± 0.31	158.89 ± 8.56
20	+	−	0	1.72 ± 0.26	164.61 ± 16.30
21	+	−	+	1.05 ± 0.22	144.37 ± 11.45
22	+	0	−	2.22 ± 0.65	150.99 ± 11.47
23	+	0	0	2.10 ± 0.66	181.76 ± 15.48
24	+	0	+	1.75 ± 0.26	170.64 ± 14.76
25	+	+	−	1.70 ± 0.66	136.68 ± 12.33
26	+	+	0	1.37 ± 0.44	172.86 ± 12.67
27	+	+	+	2.48 ± 0.21	129.73 ± 15.24

[a] +: factor at high level; 0: factor at medium level; −: factor at low level.
[b] T_L: lag time; J_s: permeation rate at steady state.
[c] Mean ± S.D., $n = 6$.

Table 3
Formulation of ibuprofen gel used for the evaluation of pharmacokinetics and relative bioavailability in rats

Ingredients	Concentration (%, w/w)
Ibuprofen	5.0
POE(5)cetyl/oleyl ether	10.0
Ethanol	30.0
Carbopol® 940	1.5
PEG-7 glyceryl cocoate	15.0
Triethanolamine	0.5
Water	38.0

Council for International Organizations of Medical Sciences as well as with institutional guidelines.

Hairless mouse skin was used for the skin permeation study of ibuprofen gel formulations (Uchida et al., 1993; Phillips and Michniak, 1995; Godwin et al., 2006). Hairless mouse skin was obtained from male ICR hairless mice, aged 6–10 weeks, weighing 20 ± 5 g. Mice were killed by spinal dislocation. Fresh skin was excised from the abdominal region and the adhering fat and other visceral tissue was removed carefully. The excised hairless mouse skin was stored at −20 °C and used within 1 week of harvesting. The receptor compartment of the Franz diffusion cell was 11.5 ml and the effective diffusion area was 1.77 cm^2. Isotonic phosphate buffer saline (PBS, pH 7.4) was used as the receptor medium, which was maintained at 32 ± 0.5 °C using a thermostatic water pump (WBC 1520, Jeio Tech Co., Korea) and stirred at a constant rate of 600 rpm during the experiment. At predetermined time intervals, 200 μl of the receptor medium was withdrawn and replaced with an equal volume of freshly prepared medium. The cumulative amounts of ibuprofen permeated through hairless mice skins were plotted as a function of time, and permeation parameters were calculated using the lag time method (Barry, 1983). The amounts of ibuprofen permeated through skin into the receptor medium were determined using a validated HPLC method as described in Section 2.6.

2.5. Pharmacokinetics study of ibuprofen gel

The optimized formulation and two marketed products, Senterlan® cream (Unicorn Laboratories, Hong Kong) and Ibutop® cream (Deutsche Chefaro Pharma GmbH, Waltrop, Germany) were topically applied to the rats in order to identify the pharmacokinetic properties and relative bioavailability of ibuprofen for all the formulations. Male Sprague–Dawley rats weighing 280 ± 20 g were used and food and water were not restricted during the study. One day before the experiment, the jugular vein of each rat was cannulated with Silastic® tubing (Dow Corning Co., U.S.A.) and polyethylene tubing (PE-50, Medichem, U.S.A.) under light diethyl ether anesthesia. Dorsal area hair was removed carefully with an electric clipper (Model 808, Daito Electric Co., Japan), then 8.6 mg/kg of each test preparation was uniformly applied to 2 cm × 3 cm of the dorsal area with gentle rubbing. After the application, 250 μL blood samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36 and 48 h postdose from the jugular vein of the rat. The collected blood samples were immediately transferred into 0.25 mL polypropylene tubes containing a small amount of heparin sodium and centrifuged at 7000 rpm for 10 min using a microcentrifuge (Model Micro 17R, Hanil Science Industrial Co., Ltd., Korea) to obtain plasma. The plasma was stored at −20 °C until analysis. For the HPLC-column switching system (CSS-HPLC), plasma samples were filtered through a 0.22-μm membrane filter (Millex® GV4, Millipore, U.S.A.) and 50 μL were directly injected onto the column without any other pre-treatment. The CSS-HPLC analysis conditions applied are described in Section 2.6.

2.6. Drug analysis

2.6.1. Analysis of ibuprofen in receptor medium using HPLC

HPLC analysis was performed using a system constructed from an isocratic pump (Waters, Model 600E, U.S.A.), an autoinjector (Waters, Model 715, U.S.A.), a UV/Vis detector (Waters, Model 486, U.S.A.) set at 223 nm and an integrator (Waters, Model IEEE-488, U.S.A.). The column used was a C_{18} column (Inertsil ODS-2, 4.6 mm × 150 mm, 5 μm particle size, GL Sciences Inc., Japan). The mobile phase was a mixture of acetonitrile and 0.2% (v/v) phosphoric acid (70:30%, v/v) with triethyl amine added at a concentration of 2×10^{-3} M. The flow rate was 1.0 mL/min. After the receptor medium was mixed with an equal volume of the internal standard solution (IS, oxaprozin 50 μg/mL in mobile phase), 50 μL of the mixed sample were injected onto the column.

2.6.2. Analysis of ibuprofen in rat plasma using CSS-HPLC

A validated CSS-HPLC method was used to determine the content of ibuprofen (Kang et al., 1998). The CSS-HPLC consisted of a Nanospace SI-1 pump (Shiseido, Tokyo, Japan) indicated as pump 1, a Hitachi L-6000 pump (Hitachi, Tokyo, Japan) as pump 2, a column oven (Waters, MA, U.S.A.), a Hitachi L-40000 UV detector, and a Nanospace SI-1 column-switching system (Shiseido, Tokyo, Japan). The column-switching system was based on the follow-

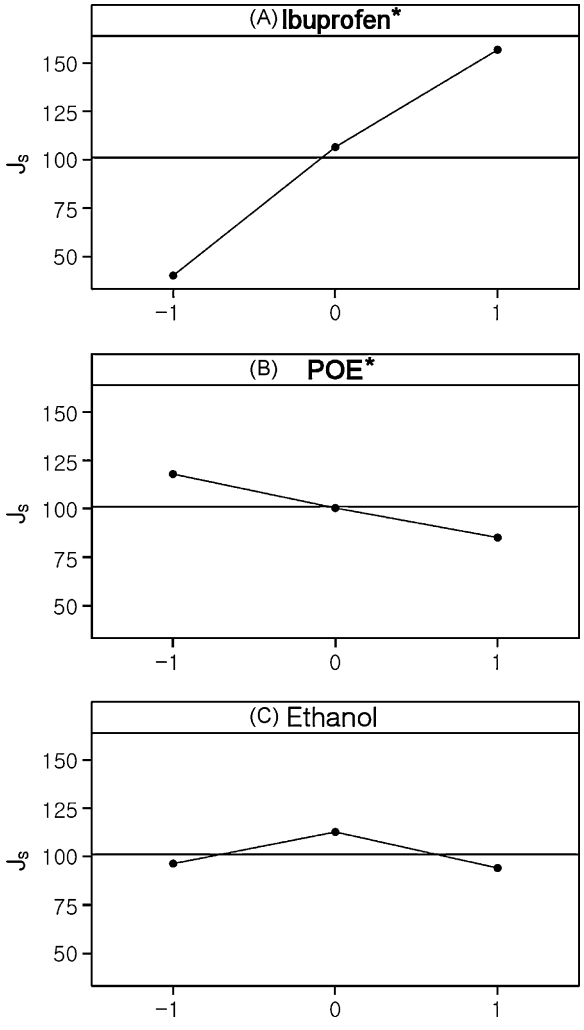

Fig. 1. Main effects plots of (A) ibuprofen, (B) POE(5)cetyl/oleyl ether and (C) ethanol concentration for J_s (*significantly different from analyzing factorial design, $p < 0.05$).

ing processes. First, the plasma was deproteinized and fractionated through the PCMF column (CAPCELL PAK® MF, 150 mm × 4.6 mm I.D., 5 μm particle size, Shiseido, Tokyo, Japan), and concentrated in the intermediate column (CAPCELL PAK® C_{18}, 35 mm × 4.6 mm I.D., 5 μm particle size, Shiseido, Tokyo, Japan). Second, the concentrated ibuprofen was transferred to the main column (CAPCELL PAK® C_{18}, 250 mm × 4.6 mm I.D., 5 μm particle size, Shiseido, Tokyo, Japan) and separated at 40 °C. The mobile phase 1 used for the PCMF column was a mixture of 50 mM sodium phosphate buffer (pH 7.0) and acetonitrile (95:5, v/v), and the mobile phase 2 used for the intermediate column and the main column was a mixture of 50 mM sodium phosphate buffer (pH 7.0) and acetonitrile (73:27, v/v). The flow rate was 1.0 mL/min and the detection was performed by monitoring the absorbance at 223 nm. Signals were collected and analyzed by the Autochro-Win Chromatography data system (Young Lin Instrument Co., Korea).

2.7. Calculations of the pharmacokinetic parameters

AUC_{0-48} is the area under the plasma concentration vs. time curve, calculated using the trapezoidal rule for the time interval 0 to the last measurable point, 48 h. The peak plasma concentration (C_{max}) and time to reach the maximum drug plasma concentration (T_{max}) was determined from visual inspection of the concentration–time plots. The relative bioavailability of optimized formulation, compared to Ibutop® cream or Senterlan® cream, was calculated according to the formula:

$$\text{Relative bioavailability}\,(\%) = \frac{[\text{AUC}]_T}{[\text{AUC}]_R} \times 100$$

where $[AUC]_R$ is the area under the curve of Ibutop® cream or Senterlan® cream, and $[AUC]_T$ is the area under the curve of optimized ibuprofen gel.

2.8. Statistics

Each experiment was repeated six times and the mean value with standard deviation was presented. Student's *t*-test was performed to see any significant difference in pharmacokinetic parameters between the control and test preparations.

3. Results and discussion

3.1. Assay validation

A previous reported HPLC assay was used to determine the content of ibuprofen in receptor medium (Park et al., 2000). The calibration curves for ibuprofen were linear at concentrations ranging from 1 to 200 μg/mL ($r > 0.999$). In order to validate the ibuprofen analysis method using HPLC, the accuracy and precision were determined as the absolute % deviation and coefficient of variation (% CV), respectively. All the validation parameters were within 9%.

A previously reported CSS-HPLC assay (Kang et al., 1998) was used to determine the content of ibuprofen in rat plasma and the calibration curves for ibuprofen were linear at concentrations ranging from 0.1 to 250 μg/mL ($r > 0.999$). All the validation parameters were also within 9% and the limit of detection for CSS-HPLC was 25 ng/mL.

The analysis methods were thus found to be precise, selective and reproducible.

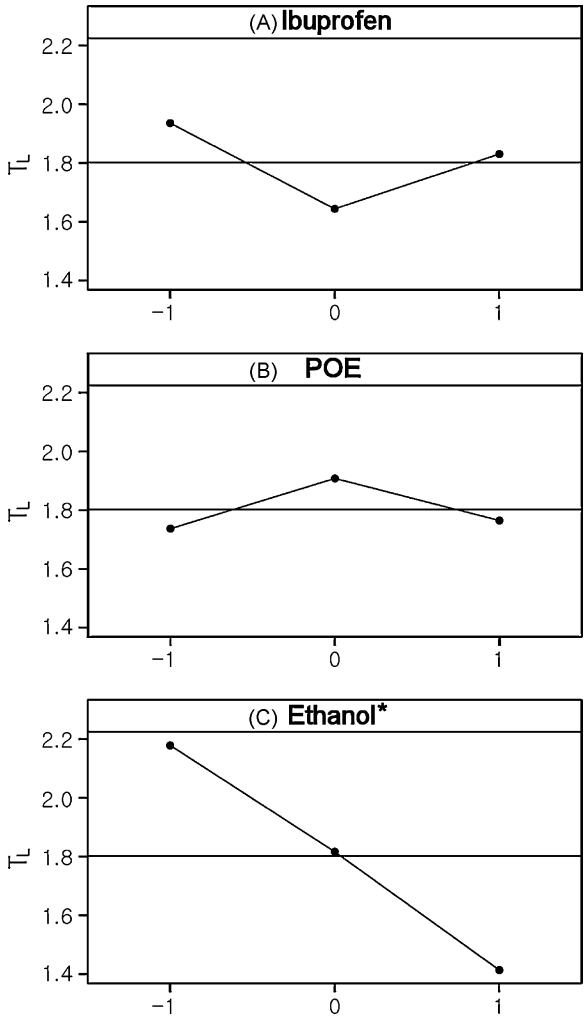

Fig. 2. Main effects plots of (A) ibuprofen, (B) POE(5)cetyl/oleyl ether and (C) ethanol concentration for T_L (*significantly different from analyzing factorial design, $p < 0.05$).

3.2. Effect of drug concentration on the skin permeation of ibuprofen

Table 2 shows the skin permeation rates (J_s) and lag times (T_L) of ibuprofen through hairless mouse skins from ibuprofen gel formulations. The main effects plot of ibuprofen concentration for J_s, which was analyzed from factorial design, is shown in Fig. 1(A). As can be seen, the permeation rate of ibuprofen significantly increased in proportion to the drug concentration. The analyzed factorial design results showed that the influences of the drug concentration on J_s were significant ($p < 0.05$). However, the effect of drug concentration on T_L was not significant (Fig. 2(A)).

3.3. Effect of POE(5)cetyl/oleyl ether on the skin permeation of ibuprofen

Our previous study showed that the enhancers containing ethylene oxide chain length of 2-5, HLB value 7–9 and an alkyl chain length C16–C18 can be the very effective promoters for the skin permeation of ibuprofen (Park et al., 2000). POE alkyl ethers are thought to enhance the penetration of a drug via both the lipophilic and the hydrophilic molecular mechanisms, and to disrupt the lipid arrangements in the stratum corneum and to increase the water content of the proteins in the barrier (Breuer, 1979; Walters et al.,

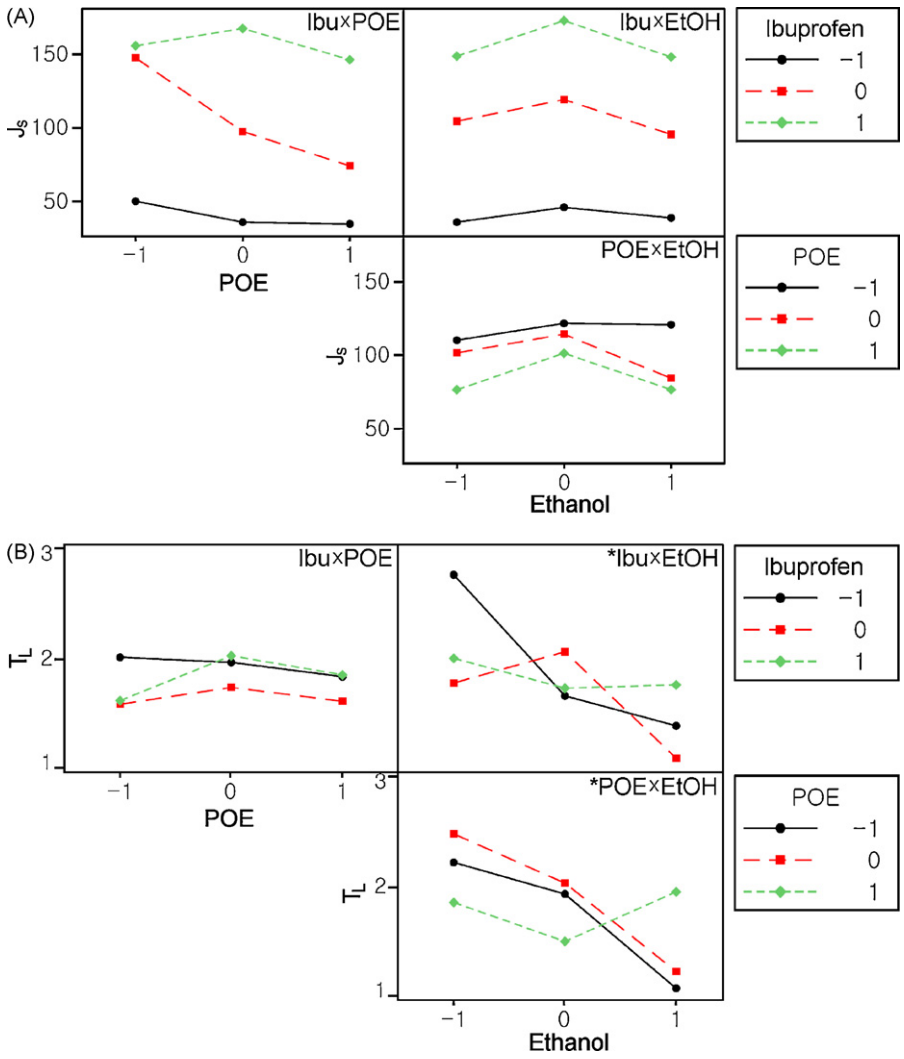

Fig. 3. Interaction plots of (A) data means for J_s and (B) data means for T_L (*significantly different from analyzing factorial design, $p < 0.05$).

1987). The structures of POE alkyl ethers are relevant to this role. However, J_s of ibuprofen significantly decreased in proportion to POE(5)cetyl/oleyl ether concentration (Fig. 1(B)). The analyzed factorial design results showed that the effects of the POE(5)cetyl/oleyl ether concentration on J_s were significant ($p < 0.05$). Penetration through the skin can be modified by surfactants, which can penetrate and interact with skin. Interaction of surfactant with the permeant (e.g., micellar solubilisation) can also occur. The permeability of ibuprofen decreased as a function of POE(5)cetyl/oleyl ether concentration probably resulting from an increase in size and number of micelles. The flux of ibuprofen through the skin was directly proportional to the concentration of free drug in the vehicle. Therefore, this observed effect was due to a decrease in thermodynamic activity as a result of micellar complexation

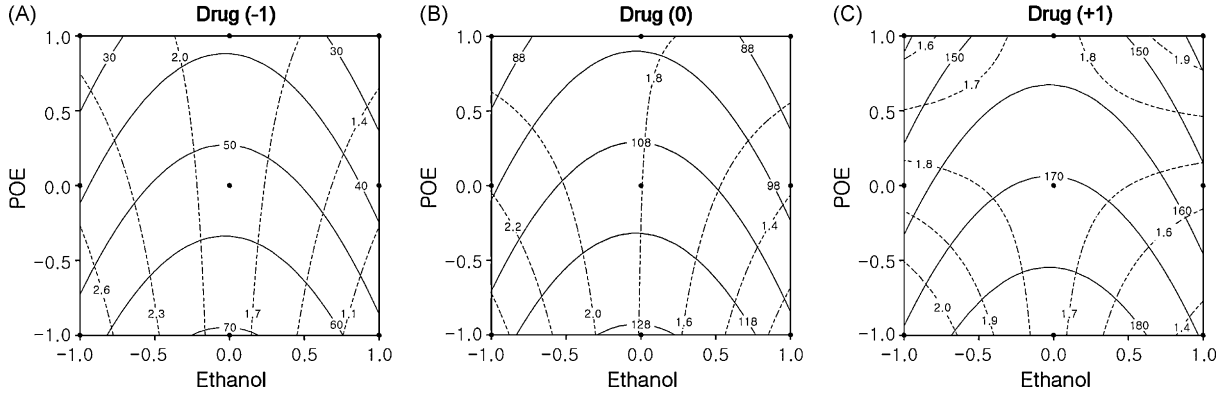

Fig. 4. Overlaid contour plots for J_s and T_L as a function of ethanol and POE(5)cetyl/oleyl ether. Key—solid line: plots of J_s; dashed line: plots of T_L.

(Cappel and Kreuter, 1991). The effect of POE(5)cetyl/oleyl ether concentration on T_L was insignificant (Fig. 2(B)).

3.4. Effect of ethanol on the skin permeation of ibuprofen

The effects of ethanol on permeation were investigated since ethanol has a significant skin penetration enhancer effect (Krishnaiah et al., 2002, 2005). However, the analyzed factorial design results showed that the effects of ethanol concentration on J_s were not significant in this study (Fig. 1(C)). The main effects plot of ethanol concentration on T_L is shown in Fig. 2(C). T_L of ibuprofen significantly decreased in proportion to ethanol concentration ($p < 0.05$).

The interaction plots of ibuprofen, ethanol and POE(5)cetyl/oleyl ether concentration on J_s and T_L, are shown in Fig. 3. The interaction plots of J_s showed that there were no significant interactions between the three terms (drug, POE(5)cetyl/oleyl ether and ethanol). In the case of T_L, the interaction between drug term and POE(5)cetyl/oleyl ether term was not significant. The interactions between the other terms (drug × ethanol, POE(5)cetyl/oleyl ether × ethanol) were significant ($p < 0.05$) because ethanol concentration had a great influence on lag time. Higher level of ethanol decreased the lag time of drug, however, medium level of ethanol was shown the shortest lag time in the case of high level of drug or POE(5)cetyl/oleyl ether.

3.5. Optimization of ibuprofen gel formulation

Response surface designs were used in this study in order to optimize the transdermal gel formulation containing ibuprofen. The statistical analysis was performed using MINITAB 14 software (Minitab Inc., PA, U.S.A.) with general optimization techniques. A computer optimization technique, based on response surface methodology, has been proven to be a useful approach for selecting pharmaceutical formulations (Takayama and Nagai, 1989; Giannakou et al., 1995; Agyralides et al., 2004; Li et al., 2005).

Fig. 4 shows the overlaid contour plots of response surface for J_s and T_L accompanying drug concentration. The maximum J_s could be achieved in the lower middle area and the minimum T_L in the lower right area at each drug concentration. The optimized formulation of ibuprofen gel was determined considering the maximal J_s and the minimal T_L—30% ethanol and 10% POE(5)cetyl/oleyl ether at each drug concentration. Formulation no. 12 (5% ibuprofen, 30% ethanol and 10% POE(5)cetyl/oleyl ether) was selected as the optimized formulation for the pharmacokinetic study. The experimental J_s and T_L of the optimized formulation were 164.45 μg/(cm^2 h) and 1.15 h, respectively.

3.6. Pharmacokinetic study of ibuprofen gel

Plasma drug concentration vs. time profiles of ibuprofen after topical administration of the optimized formulation and two marketed products to six rats are shown in Fig. 5. Pharmacokinetic parameters calculated from the plasma drug concentration vs. time profiles are listed in Table 4. There were significant differences in AUC_{0-48} and C_{max} between the optimized ibuprofen gel and the cream formulations, whereas there was no difference in T_{max}. The C_{max} of the optimized ibuprofen gel is over 1.7 times greater than that of Senterlan® cream and 3.5 times greater than Ibutop® cream. After topical administration, ibuprofen levels in the plasma quickly reached detectable levels; however, C_{max} fell soon after reaching therapeutic plasma levels. This is a good indication that the topical administration of ibuprofen does not lead to systemic adverse effects (Berner et al., 1989). The optimized ibuprofen gel had a relative bioavailability of 228.8% and 181.0% compared to Ibutop® cream and Senterlan® cream, respectively.

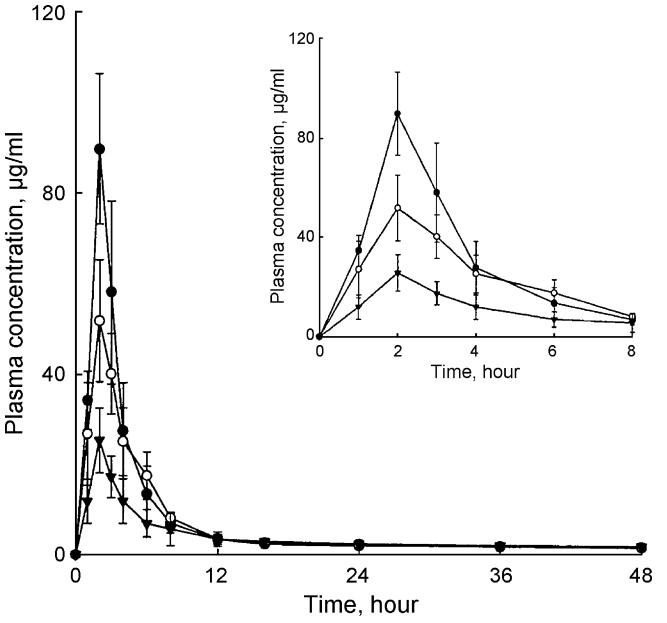

Fig. 5. Plasma concentration–time profiles following transdermal application of ibuprofen gel, Senterlan® cream and Ibutop® cream on dorsal skin of rats (mean ± S.D., $n = 6$). Key: (○) ibuprofen gel; (●) Senterlan® cream; (▼) Ibutop® cream.

Table 4
Pharmacokinetic parameters of ibuprofen gel and control preparations following transdermal application on the dorsal skin region of rats

Parameters	Preparations		
	Ibuprofen gel	Senterlan® cream	Ibutop® cream
Dose (mg/kg)	8.6	8.6	8.6
T_{max} (h)	2.0 ± 0.0[a]	2.0 ± 0.0	2.0 ± 0.0
C_{max} (μg/mL)	89.8 ± 16.7*	51.7 ± 13.3	25.4 ± 7.2
AUC_{0-48} (μg h/mL)	285.3 ± 47.5*	157.6 ± 26.3	124.7 ± 20.8
Relative BA[b] (%)	181.0	100.0	–
	228.8	–	100.0

[a] Mean ± S.D., $n = 6$.
[b] Relative bioavailability compared to reference formulation.
* Significantly different from the control preparations ($p < 0.05$).

In conclusion, transdermal ibuprofen gels were formulated successfully using experimental design techniques and these results suggest that the experimental design technique is an effective tool to develop pharmaceutical formulations.

References

Agyralides, G.G., Dallas, P.P., Rekkas, D.M., 2004. Development and in vitro evaluation of furosemide transdermal formulations using experimental design techniques. Int. J. Pharm. 281, 35–43.
Al-Saidan, S.M., Krishnaiah, Y.S., Chandrasekhar, D.V., Lalla, J.K., Rama, B., Jayaram, B., Bhaskar, P., 2004. Formulation of an HPMC gel drug reservoir system with ethanol-water as a solvent system and limonene as a penetration enhancer for enhancing in vitro transdermal delivery of nicorandil. Skin Pharmacol. Physiol. 17, 310–320.
Babar, A., Solanki, U.D., Cutie, A.J., Plakogiannis, F., 1990. Piroxicam release from dermatological bases: in vitro studies using cellulose membrane and hairless mouse skin. Drug Dev. Ind. Pharm. 16, 523–540.
Barry, B.W., 1983. Dermatological Formulations. Marcel Dekker Inc., NY, pp. 49–94.
Berner, G., Engels, B., Vögtle-Junkert, U., 1989. Percutaneous ibuprofen therapy with Trauma-Dolgit gel: bioequivalence studies. Drug. Exp. Clin. Res. 15, 559–564.
Brown, M.B., Hanpanitcharoen, M., Martin, G.P., 2001. An in vitro investigation into the effect of glycosaminoglycans on the skin partitioning and deposition of NSAIDs. Int. J. Pharm. 225, 113–121.

Breuer, M.M., 1979. The interaction between surfactants and keratinous tissues. J. Soc. Cosmet. 30, 41–64.
Busson, M.J., 1986. Update on ibuprofen: review article. J. Int. Med. Res. 14, 53–62.
Cappel, M.J., Kreuter, J., 1991. Effect of non-ionic surfactants on transdermal drug delivery. I. Polysorbates. Int. J. Pharm. 69, 143–153.
Chang, S.Y., Park, S.B., Jung, J.H., Shon, S.I., Yoon, H.J., 1997. Iburpofen heterocyclic esters as dermal prodrugs: in vitro evaluation. S.T.P. Pharma Sci. 7, 315–319.
Chen, H., Chang, X., Du, D., Li, J., Xu, H., Yang, X., 2006. Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. Int. J. Pharm. 315, 52–58.
Cilurzo, F., Minghetti, P., Casiraghi, A., Tosi, L., Pagani, S., Montanari, L., 2005. Polymethacrylates as crystallization inhibitors in monolayer transdermal patches containing ibuprofen. Eur. J. Pharm. Biopharm. 60, 61–66.
Fang, C., Liu, Y., Ye, X., Rong, Z.X., Feng, X.M., Jiang, C.B., Chen, H.Z., 2008. Synergistically enhanced transdermal permeation and topical analgesia of tetracaine gel containing menthol and ethanol in experimental and clinical studies. Eur. J. Pharm. Biopharm. 68, 735–740.
Giannakou, S.A., Dallas, P.P., Rekkas, D.M., Choulis, N.H., 1995. Development and in vitro evaluation of nitrendipine transdermal formulations using experimental design techniques. Int. J. Pharm. 125, 7–15.
Godwin, D.A., Wiley, C.J., Felton, L.A., 2006. Using cyclodextrin complexation to enhance secondary photoprotection of topically applied ibuprofen. Eur. J. Pharm. Biopharm. 62, 85–93.
Gupta, R.R., Singh, K.K., 2005. Polyoxyethylene alkyl ethers. In: Rowe, R.C., Sheskey, P.J., Owen, S.C. (Eds.), Handbook of Pharmaceutical Excipients, 5th ed. American Pharmacists Association, Washington, DC, USA/The Pharmaceutical Press, Royal Pharmaceutical Society of Great Britain, London, England.
Hamed, E., Sakr, A., 2001. Application of multiple response optimization technique to extended release formulations design. J. Control. Rel. 73, 329–338.
Iervolino, M., Cappello, B., Raghavan, S.L., Hadgraft, J., 2001. Penetration enhancement of ibuprofen from supersaturated solutions through human skin. Int. J. Pharm. 212, 131–141.
Kang, S.H., Chang, S.Y., Do, K.C., Chi, S.C., Chung, D.S., 1998. High-performance liquid chromatography with a column-switching system and capillary electrophoresis for the determination of ibuprofen in plasma. J. Chromatogr. B Biomed. Sci. Appl. 712, 153–160.
Krishnaiah, Y.S., Satyanarayana, V., Bhaskar, P., 2002. Influence of limonene on the bioavailability of nicardipine hydrochloride from membrane-moderated transdermal therapeutic systems in human volunteers. Int. J. Pharm. 247, 91–102.
Krishnaiah, Y.S., Al-Saidan, S.M., Chandrasekhar, D.V., Satyanarayana, V., 2005. Bioavailability of nerodilol-based transdermal therapeutic system of nicorandil in human volunteers. J. Control. Rel. 106, 111–122.
Li, W., Nadig, D., Rasmussen, H.T., Patel, K., Shah, T., 2005. Sample preparation optimization for assay of active pharmaceutical ingredients in a transdermal drug delivery system using experimental designs. J. Pharm. Biomed. Anal. 37, 493–498.
Lopez, V.C., Raghavan, S.L., Snowden, M.J., 2004. Colloidal microgels as transdermal delivery systems. React. Funct. Polym. 58, 175–185.
Muktadir, A., Babar, A., Cutie, A.J., Plakogiannis, F.M., 1986. Medicament release from ointment bases. III. Ibuprofen: in vitro release and in-vivo absorption in rabbits. Drug Dev. Ind. Pharm. 12, 2521–2540.
Park, E.S., Chang, S.Y., Hahn, M., Chi, S.C., 2000. Enhancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. Int. J. Pharm. 209, 109–119.
Phillips, C.A., Michniak, B.B., 1995. Transdermal delivery of drugs with differing lipophilicities using azone analogs as dermal penetration enhancers. J. Pharm. Sci. 84, 1427–1433.
Ranman, M.S., Babbar, A., Patel, N.K., Plakogiannis, F.M., 1990. Medicament release from ointment bases: V. Naproxen in vitro release and in vivo percutaneous absorption in rabbits. Drug Dev. Ind. Pharm. 16, 651–672.
Swart, H., Breytenbach, J.C., Hadgraft, J., du Plessis, J., 2005. Synthesis and transdermal penetration of NSAID glycoside esters. Int. J. Pharm. 301, 71–79.
Takahashi, A., Suzuki, S., Kawasaki, N., Kubo, W., Miyazaki, S., Loebenberg, R., Bachynsky, J., Attwood, D., 2002. Percutaneous absorption of non-steroidal anti-inflammatory drugs from in situ gelling xyloglucan formulations in rats. Int. J. Pharm. 246, 179–186.
Takayama, K., Nagai, T., 1989. Novel computer optimization methodology for pharmaceutical formulations investigated by using sustained-release granules of indomethacin. Chem. Pharm. Bull. 37, 160–167.
Uchida, T., Lee, C.K., Sekiya, N., Goto, S., 1993. Enhancement effect of an ethanol/Panasate 800 binary vehicle on anti-inflammatory drug permeation across excised hairless mouse skin. Biol. Pharm. Bull. 16, 168–171.
Walters, K.A., Walker, M., Olejnik, O., 1987. Non-ionic surfactant effects on hairless mouse skin permeability characteristics. J. Pharm. Pharmacol. 40, 525–529.