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Formulation optimization of meloxicam sodium gel using response surface methodology

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Abstract

The influences of a combination of different mechanisms of penetration enhancers on the penetration absorption properties of meloxicam sodium formulations through rat skin were investigated using response surface methodology. A uniform design was applied to prepare model formulations systematically that were composed of four independent variables: the content of ethanol (x_1), propylene glycol (x_2), menthol (x_3), and azone (x_4). The penetration rate (flux) of meloxicam sodium gel through rat skin was chosen as the response which had to be higher than 400 μg/h cm^2 the required flux of meloxicam gel to maintain a therapeutic concentration. The result showed optimal formulation could be obtained from this response surface methodology. Menthol had the greatest potential influence on the penetration absorption of meloxicam sodium, followed by azone, ethanol and PG, respectively. By in vivo study, meloxicam could be determined 1 h after topical administration and reached steady-state concentration at about 12 h. The bioavailability (%) of the optimal meloxicam sodium gel was about 50.1%.

Keywords: Meloxicam sodium; Menthol; Azone; Response surface methodology; Uniform design

1. Introduction

Meloxicam is a potent, nonsteroidal anti-inflammatory drug approved by FDA in 2000 in treatment of arthritis, osteoarthritis and degenerative joint disease (Engerlhardt, 1996; Noble and Balfour, 1996; Stei et al., 1996; Busch et al., 1998; Kaplan-Machlis and Klostermeyer, 1999). In view of the characteristics of meloxicam including small oral dosage (7.5–15 mg/day), low molecular weight (354.1), lipid solubility and excellent tissue tolerability (Parfitt, 1999), it seems that there is potential for investigating the meloxicam transdermal delivery system as an additional route for meloxicam administration (Stei et al., 1996; Gupta et al., 2002; Chang et al., 2006). Meloxicam performs very poorly in aqueous solubility and wettability, leading to difficulties in the design of pharmaceutical formulations. In order to overcome these problems, meloxicam sodium was used as the model drug in this study.

The most difficult aspect of the transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. It is well known that the penetration rate of drugs through the stratum corneum can be increased with appropriate vehicles and transdermal penetration enhancers, owing to their ability to increase the solubility of drug and/or enhancers in pharmaceutical formulations and to change the structure of lipophilic and/or keratinized domains in stratum corneum (Lambert et al., 1989; Ashton et al., 1992; Morimoto et al., 1993; Kabayashi et al., 1994; Wu et al., 2001). In the development of transdermal dosage form, an important issue was to design an optimized pharmaceutical formulation with appropriate penetration rate within a short time period and minimum trials. For this purpose, a computer optimization technique based on

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a response surface methodology (RSM) utilizing polynomial equations and artificial neural networks (ANN) has been widely used (Takayama and Nagai, 1989; Sonaglio et al., 1995; Huang et al., 2004). The optimization procedure involved systematic formulation designs to minimize the number of trials, and analyze the response surfaces in order to realize the effect of causal factors and to obtain the appropriate formulations with target goals. Therefore, in order to quickly obtain the optimal formulations with appropriate penetration rate of meloxicam sodium transdermal delivery system, a computer optimization technique based on an RSM (Takayama and Nagai, 1989; Huang et al., 2004), was used to evaluate the effects of different types and mechanisms of vehicles and transdermal penetration enhancers at various concentrations on drug penetration rate through rat skin. Furthermore, the pharmacokinetic parameters of the optimal formulation of meloxicam sodium were also determined by the occlusion dressing technique in rats.

2. Materials and methods

2.1. Materials

The following reagents were used: meloxicam sodium, piroxicam (Sigma Chemical Company, USA), hydroxypropyl cellulose (HPC 1000-4000), azone, menthol and propylene glycol (PG) (Tokyo Chemical Industry, Japan). All other chemicals and solvents were of analytical reagent grade.

2.2. Solubility measurement

An excess of meloxicam sodium was placed in contact with 2 mL of solvent in sealed glass tubes. The tubes were shaken occasionally on a vortex mixer and were maintained at room temperature for 24 h. The saturated solution was centrifuged and the supernatant was filtered through a 0.45 μm membrane. The concentration of drug in the saturated solution was determined by HPLC after appropriate dilution with the selected solvents.

2.3. Preparation of meloxicam gels

Uniform design was developed by Fang in 1980 by combining the theory of numbers and multivariable statistics. In the uniform design, each factor and each level only needs one test and the number of tests is equal to that of levels, since there are fewer design points and fewer experiments to be performed in comparison with other orthogonal designs such as factorial design and central composite design (Liu et al., 1993; Sheng et al., 2005; Zheng and Bi, 2005). Hence, the uniform design was applied to prepare systematical model formulations, which were composed of four formulation factors: the content of ethanol (x_1), PG (x_2), and menthol (x_3) and azone (x_4). The factors and levels were arranged according to the rule of the uniform design. The range of each independent variable was predetermined using preliminary experiments. The compositions of all model formulations are summarized in Table 1. The penetration rate (flux) was chosen as the response.

Meloxicam and transdermal enhancers were dissolved in the mixture of pH 7.4 buffer solution containing ethanol and PG. The hydroxypropyl cellulose was added and maintained at RT overnight. Then, all components were mixed well while they were completely hydrated. The hydrogels were stored in air-tight containers at room temperature.

2.4. In vitro skin permeation experiments

The extent and rate of skin permeation of meloxicam sodium from gel formulations were determined using a modified glass diffusion cell fitted with excised rat skin. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor cell was filled with 1 g of 1% meloxicam gel and occluded by paraffin. The receptor compartment was filled with 20 mL of pH 7.4 phosphate buffer containing 12.5% (w/v) ethanol and 12.5% (w/v) PG and its temperature was maintained at 37 ± 0.5 °C by thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm^2. Approximately 0.5 mL of the recep-

Table 1
The level, composition and responses of meloxicam sodium model formulations using uniform design

	x_1 (%, w/v)	x_2 (%, w/v)	x_3 (%, w/v)	x_4 (%, w/v)	Response flux (μg/cm^2 h)
C 01	10.0	25	0.0	0.0	4.55 ± 1.02
C 02	25.0	10	0.0	0.0	3.99 ± 1.37
Run 01	15.0	17	2.0	0.5	80.43 ± 36.39
Run 02	17.5	9	4.5	1.5	190.66 ± 28.12
Run 03	20.0	23	1.5	2.5	158.96 ± 3.32
Run 04	22.5	15	4.0	3.5	34.63 ± 15.23
Run 05	25.0	7	1.0	4.5	52.96 ± 8.33
Run 06	27.5	21	3.5	0.0	315.79 ± 59.19
Run 07	30.0	13	0.5	1.0	104.38 ± 23.56
Run 08	32.5	5	3.0	2.0	193.35 ± 36.00
Run 09	35.0	19	0.0	3.0	397.33 ± 88.13
Run 10	37.5	11	2.5	4.0	442.00 ± 66.49
Run 11	40.0	25	5.0	5.0	307.95 ± 65.15

The amount of meloxicam sodium and HPC were fixed at 1% (w/v) and 2.5% (w/v), respectively.

tor medium was withdrawn at determined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the penetration data. The sample withdrawn from the receptor compartment was then analyzed by HPLC as described in a previous study (Chang et al., 2006). A Merck Lichrocart® C18 column (55 mm × 4 mm i.d., particle size 3 μm) was used. The mobile phase was a mixture of 0.05 M di-ammonium phosphate (adjusted to pH 6.5 by phosphoric acid) and methanol in the ratio of 55:45, at the flow rate of 1 mL/min. The UV detection was at 365 nm. The limit of detection was 0.025 g/mL (signal-to-noise > 4). Each data point represents the average of three determinations.

2.5. Statistical analysis of data

The cumulative amount of the drug permeation through rat skin was plotted as a function of time and linear regression analysis was used to calculate the penetration rate (flux) of drug.

The flux (response) and formulation variables of all model formulations were treated by Design-Expert® software. Statistical analysis including stepwise linear regression and response surface analysis were conduced. The significant terms ($p < 0.005$) were chosen for final equations. Suitable models for uniform designs consisting of three components include linear, quadratic and special cubic models. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient of variation (c.v.), the multiple correlation coefficient (R^2), and adjusted multiple correlation coefficient (adjusted R^2) proved by Design-Expert software (Huang et al., 2004).

2.6. In vivo absorption study

Wistar rats weighting about 230–250 g were used in the pharmacokinetics study in accordance with a protocol approved by the Institutional Review Board-Use and Care of Animals at Kaohsiung Medical University. These animals were divided into two groups for IV bolus and topical application administration. Rats were anesthetized with urethane (1.2 g/kg) by intraperitoneal injection. Meloxicam sodium solution (20 mg/kg) and gel (1.5 mL) were administered via the tail vein and shaven abdomen by the occlusive dressing technique, respectively. Blood samples (0.3 mL) were drawn from jugular vein at the appropriate intervals. Each blood sample was centrifuged for 10 min at 3000 rpm. The plasma sample 0.1 mL was placed into a tube containing 5 mL of dichloromethane, 0.02 mL of 2N HCl and 0.02 mL of 5 mg/mL piroxicam. The mixture was shaken horizontally for 10 min and centrifuged at 3000 rpm for 10 min. The organic layer was transferred to another tube and evaporated to dryness by vacuum pump. The dry residue was reconstituted in 0.2 mL of mobile solution and the clear supernatant was injected into the HPLC system.

Pharmacokinetic parameters for meloxicam sodium in rat were measured using a two-compartment model fitting by utilizing the least-square fit program (WinNonlin software, USA).

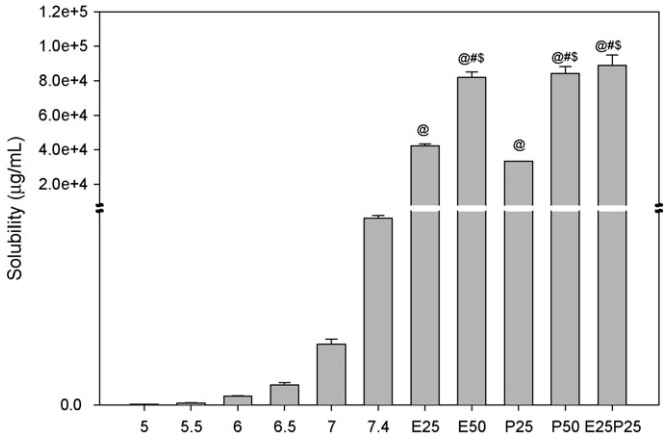

Fig. 1. Solubility of meloxicam sodium in different pH value buffer and combination of pH 7.4 buffer, ethanol and PG (E25: 25% ethanol; E50: 50% ethanol; P25: PG 25%; P50: PG 50%; E25P25: ethanol 25%; PG 25% in pH 7.4 buffer). [@]Significant difference compared with pH 7.4 buffer; [#]significant difference compared with pH 7.4 buffer containing 25% ethanol; [$]significant difference compared with pH 7.4 buffer containing 25% PG.

3. Results and discussion

3.1. Solubility

As shown in Fig. 1, the solubility of meloxicam sodium was dependent on the pH value of the vehicle; the solubility increased with increase in pH value. The results were consistent with that of meloxicam free base. The use of cosolvents has been employed by a number of studies to enhance the solubility of poorly soluble drugs (Katayama et al., 2001; Seedher and Bhatia, 2003). The mall nonpolar hydrocarbon region in the cosolvent can reduce the ability of the aqueous system to squeeze out nonpolar solutes. As expected, the solubility of meloxicam sodium in pH 7.4 buffer containing cosolvent such as ethanol and PG was significantly higher (ANOVA, $p < 0.05$) than in pH 7.4 buffer alone, by a 7–18-fold increase. The solubility in 25% (w/v) ethanol, 25% (w/v) PG and the combination solvent of 25% (w/v) ethanol and 25% (w/v) PG were 42.38 ± 1.1, 33.41 ± 0.01 and 88.94 ± 0.59 mg/mL, respectively. The solubility in the combination solvent was higher than the additive of individual solubility of 25% ethanol and 25% PG, indicating that the combination of ethanol and PG had a synergistic effect on enhancement solubility of the drug.

3.2. In vitro skin permeation experiments

In our preliminary study, azone and menthol had potential enhancement on meloxicam permeation through rat skin (Chang et al., 2006). However, azone and menthol are hydrophobic drugs and need a cosolvent to help them dissolve in formulation. Additionally, some reports (Cooper, 1984; Takeuchi et al., 1995; Katayama et al., 2001) have indicated that specific combinations of vehicles and enhancers such as menthol in ethanol and azone in PG had synergistic enhancement penetration effect. Therefore, in this study, the combination of ethanol,

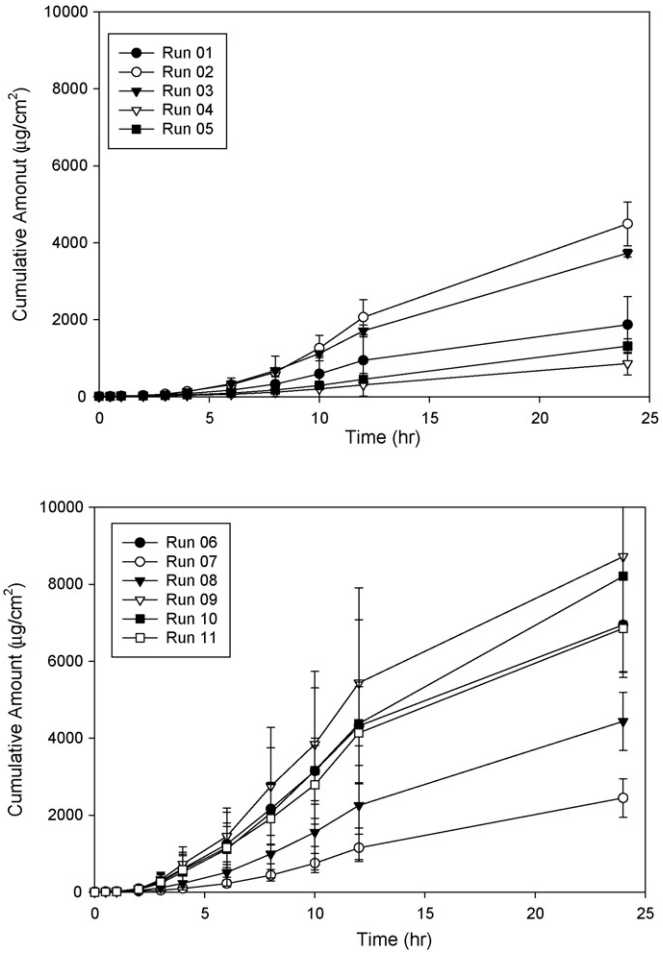

Fig. 2. In vitro penetration–time profile of meloxicam sodium model formulations through rat skin ($n = 3$).

PG, menthol and azone were used as multi-enhancers to produce the synergistic enhance effect on penetration rate of meloxicam sodium and to decrease the used amount of enhancers. In order to quickly obtain an optimal formulation with fewer experimental trials and quantify the effect of these enhancers, a computer optimization technique including uniform design (Fang, 1980; Zheng and Bi, 2005) and response surface methodology (Mayer and Montgomery, 1995; Singh et al., 1995) was used. Fig. 2 shows the permeation profiles of these meloxicam sodium model formulations through excised rat skin. The rat skin permeation profile of meloxicam sodium exhibited a zero-order permeation at a constant penetration rate ($R^2 > 0.9318$). The independent variables and response (penetration rate) of these model formulations are shown in Table 1. The responses of these model formulations ranged from a low drug penetration rate of 34.6 μg/cm^2 h (run 04, ethanol 22.5%, PG 15%, menthol 4% and azone 3.5%) to a higher penetration rate of 442.0 μg/cm^2 h (run 10, ethanol 37.5%, PG 11%, menthol 2.5% and azone 4.0%). The wide variation indicated that different enhancer combination could result in different enhancement effects on drug permeation through skin. For estimation of quantitative effects of the different combination of factors and factor levels on the flux, the response surface models were calculated with Design-Expert software by applying coded values of factor levels. The model described could be represented as:

$$\text{response} = -28.3 - 29.0x_1 + 18.0x_2 + 96.9x_3 + 61.8x_4 + 1.8x_1x_2 + 5.9x_1x_3 + 2.1x_1x_4 - 16.4x_2x_3 - 9.1x_2x_4$$

The significant p-value (significance probability value), R-square, adjusted R-square and c.v. of this model were <0.0001, 0.8493, 0.7903 and 30.7, respectively, indicated that the assumed regression model was significant and valid for each considered response. The values of the coefficients in the model are related to the effect of these variables on the response. A positive sign indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response (Hamed and Sakr, 2001). From this model, the rank order of standardized coefficient was $x_3 > x_4 > x_1 > x_2$, indicating that menthol had the greatest potential influence on the penetration absorption of meloxicam sodium, followed by azone, ethanol and PG, respectively. Additionally, the interaction effects of ethanol/menthol and ethanol/Azone also showed positive significant effects. The three-dimensional response surfaces (Fig. 3) were drawn to estimate the effects of the independent variables on response and to select the optimal formulation. Since the model has more than two factors, two factors were included at medium levels for each diagram. According to previous studies (Busch et al., 1998; Chang et al., 2006), the required flux of meloxicam gel to reach a therapeutic concentration was about 400 μg/h through rat skin. Hence, the penetration rate of optimal formulations in the optimization process was set at above 400 μg/h. Two optimal formulations were obtained from the response surfaces method, the composition and predicted responses of which are listed in Table 2. To confirm the validity of the calculated optimal parameters and predicted responses, the optimum formulations were prepared according to the above values of the factors and subjected to in vitro penetration experiments. From the results presented in Table 2, the predicted error is below 15%, indicating that the observed responses were very close to the predicted values.

Table 2
The independent variables (factors) and dependent variables (responses) of the optimal formulations

	x_1	x_2	x_3	x_4	Predicted response	Observed response	Predicted error (%)
P01	37.1	15.4	2.9	4.3	406.3	467.6 ± 89.3	−15.07
P02	35.9	20.0	4.0	2.1	440.9	431.2 ± 83.9	2.20

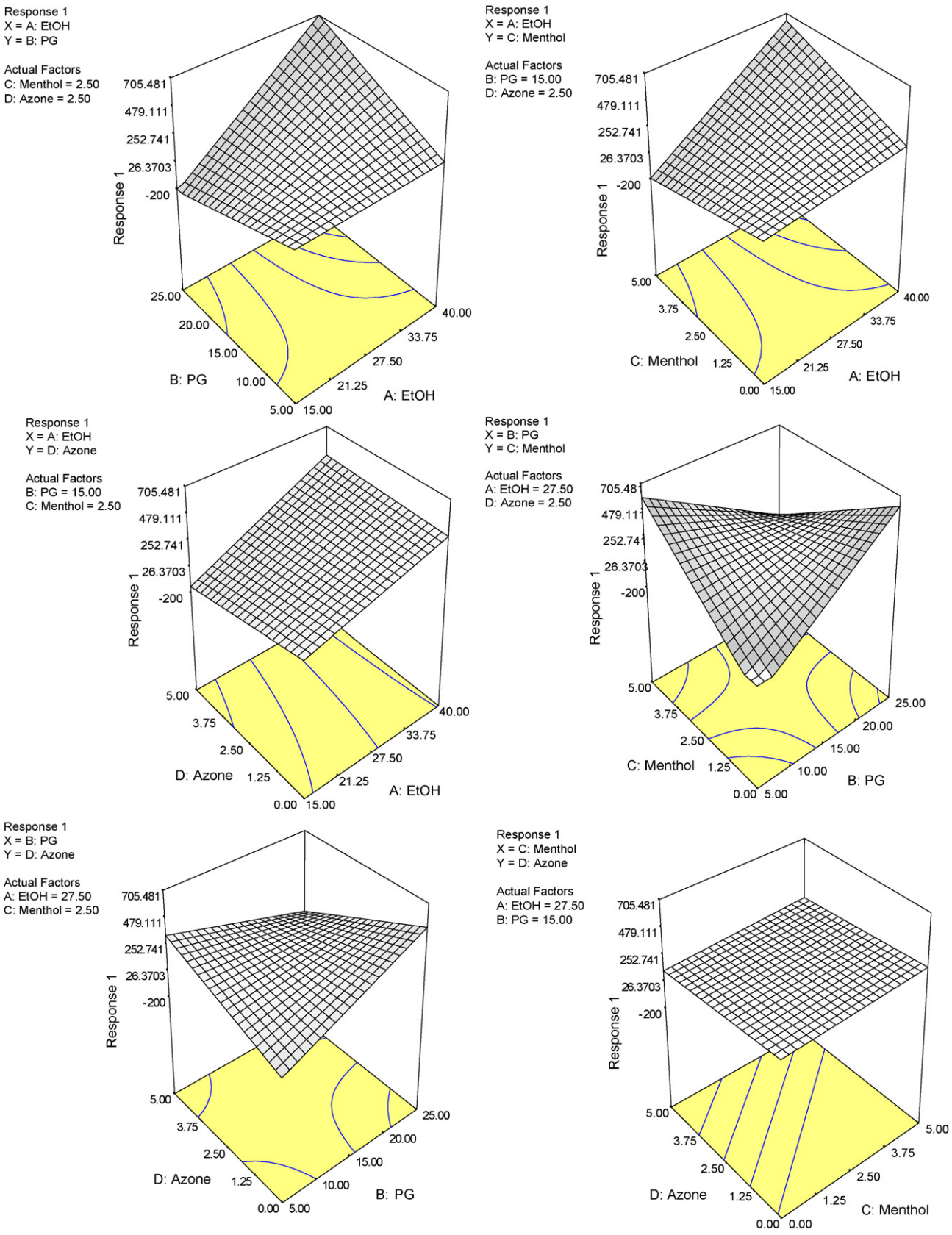

Fig. 3. Response surface plots (3D) showing the effect of four kinds of enhancers on the response (penetration flux of meloxicam sodium gel through rat skin).

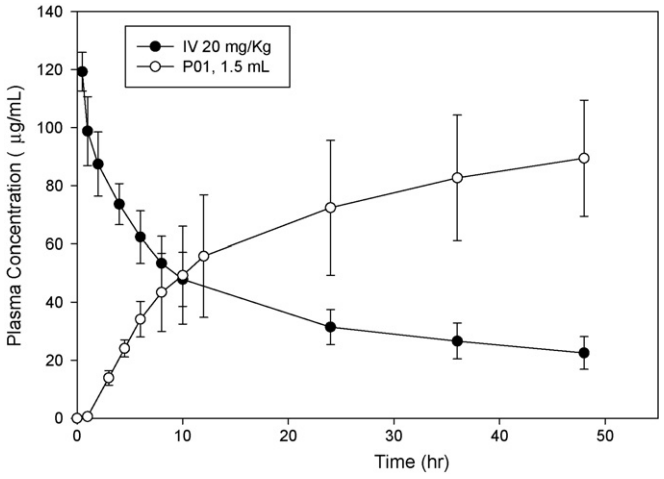

Fig. 4. Plasma concentration–time profile after IV (20 mg/kg) and topical (P01 gel formulation, 15 mg/2.3 cm^2) administration ($n-4$).

3.3. In vivo absorption study

The plasma concentration–time profiles after IV (20 mg/kg) and topical (15 mg/2.3 cm^2) administration are shown in Fig. 4. The plasma concentration of meloxicam sodium after IV administration declined in a biexponential manner. The plasma level of meloxicam sodium was adequately described using a two-compartment open model ($R^2 > 0.9921$; AIC = 65.9) by utilizing the least-square fit program. The AUC, $t_{1/2}(\beta)$ and clearance were 3054.4 ± 931.3 mg h/L, $37.8.4 \pm 8.5$ h and 0.007 ± 0.002 L/h kg, respectively. As shown in Fig. 3, meloxicam could be determined at 1 h after 2.3 cm^2 topical administration, and then the plasma concentration increased quickly with the time required to reach a steady-state concentration 46.64 ± 27.87 μg/mL at about 12 h. Gupta et al. (2002) reported that a meloxicam gel with C_{max} of 48.48 ± 6.57 μg/mL after 9 cm^2 application in rat could be an effective formulation in inflammatory conditions. Therefore, it could be concluded that the meloxicam sodium gel in the present study could be used accordingly. The bioavailability (%) through transdermal absorption was 50.1%, calculated by $(AUC_{0-48\,h}/D_{ta})/(AUC_{0-48\,h}/D_{iv})$.

4. Conclusion

The result showed that response surface methodology (RSM) including uniform design can be used successfully to design a meloxicam sodium hydrogel. The optimal transdermal formulation had appropriate flux which met the required flux of meloxicam for maintaining a therapeutic concentration. The bioavailability (%) of the meloxicam sodium gel was about 50.1%.

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