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In-Vitro Evaluation of Meloxicam Permeation Using Response Surface Methodology

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ABSTRACT

The influences of combination of different mechanisms of penetration enhancers (such as azone, sodium lauryl sulfate and menthol) on the percutaneous absorption of meloxicam formulations through rat skin were investigated using uniform deign and response surface methodology. The uniform design was applied to prepare systematic model formulations which were composed of three formulation factors: azone (x_1), sodium lauryl sulfate (x_2), and menthol (x_3). The result showed that azone had the highest potential influence on the penetration absorption of meloxicam, followed by sodium lauryl sulfate and menthol. With zero-order delivery, the required flux of meloxicam gel to maintain a therapeutic concentration was about 400 μg/hr/cm^2. The result showed that the optimal addition concentration of azone at 4% to 6% could be obtained at high penetration rate and short lag time of meloxicam gel. It was shown that as the concentration of sodium lauryl sulfate increased from 0% to 12% the flux and cumulative amount at 48 hr increased and lag time decreased. On the other hand, menthol had the effect of a shorter lag time. To validate the predictive ability of the hypothesized model, the predicted formulations were prepared and penetration experiments were performed. The predicted formulations were developed for the transdermal drug delivery system.

Key words: Meloxicam, Azone, Response surface methodology, *In-vitro* permeation

INTRODUCTION

Meloxicam is a potent, newer nonsteroidal anti-inflammatory drug approved by FDA in 2000 and is used in treatment of reheumatoid arthritis, osteoarthritis and degenerative joint disease. Although meloxicam preferentially inhibits COX-2 (cyclooxgenease-2) over COX-1 (which is responsible for physiological processes in the stomach and kidney), it still has 10-20% incidence of gastrointestinal side effect[1-6]. Meloxicam also possesses appropriate physiochemical properties for potential transdermal delivery such as low molecular weight, low polarity, low melting point and low daily therapeutic dose. The molecular weight (354.1) of meloxicam is appropriate but the aqueous solubility is poor. The oral efficiency dose (7.5-15 mg/day) of meloxicam is the lowest in the nonsteroidal anti-inflammatory drugs. Moreover, previous study[6] reported that meloxicam formulation exhibited excellent tissue tolerability and appeared to be suitable for dermal administration. Furthermore, topical administration via the dermal route can bypass disadvantages of the oral route. Therefore, the transdermal drug delivery has been considered an additional route for meloxicam administration[3,7]. The most difficult aspect of transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. The use of penetration enhancer is valuable and important for achieving therapeutic plasma levels for many drugs[8-10]. However, penetration enhancer is known to causes extensive damage to skin, along with the large increase in transdermal penetration rate by added large amount of enhancers. As a result, the appropriate penetration rate and acceptable level of irritation must be considered at the same time in design of an optimum transdermal formulation. It may be possible to maintain the penetration rate and reduce the skin irritation by incorporating several different enhancement mechanisms of enhancers in the gel formulation to create synergistic enhancement effect. Therefore, the purpose of the present study was to investigate the effects of combination of several enhancers in meloxicam hydrophilic gel on the percutaneous absorption. Azone was suggested to influence the lipid fluidizing, alter the keratin structure on stratum corneum lipids[11], and effectively enhance the permeability of many drugs[8-9,12]. Sodium lauryl sulfate (sodium dodecyl sulfate, SDS) was speculated to bind strongly with protein of stratum corneum, thus causing a reversible denaturation and an uncoiling of the filaments[13]. Enhancing effect of menthol was thought to increase the tissue partition of lipophilic drug[14-15]. Moreover, menthol has the advantage of a shorter lag time; therefore, it has been broadly used in many topical formulations containing nonsteroidal anti-inflammatory drugs to increase the pharmaceutical effects[16]. In this study, all three substances were used as penetration enhancers to enhance the percutaneous absorption of meloxicam.

In order to obtain the optimal formulation, a computer optimization technique based on a response surface methodology (RSM) utilizing polynomial equation[17-20] was used to search for the optimal meloxicam formulation

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Journal of Food and Drug Analysis, Vol. 14, No. 3, 2006

and quantify the influences of formulation variables on the drug permeation.

In the present study, the effect of several pure and mixed solvent systems on the *in vitro* skin permeability of meloxicam was investigated in order to select a solvent system as the first step towards developing a transdermal therapeutic system.

MATERIALS AND METHODS

I. *Materials*

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

II. *Preparation of Meloxicam Gels*

In order to easily optimize the formulation and evaluation of the influence of each enhancer on the percutaneous rate, the uniform design[21] was applied to prepare systematic model formulations which were composed of three formulation factors: azone (x_1), SDS (x_2), and menthol (x_3). The factors and levels were arranged according to the Uniform Design. The compositions of all model formulation are summarized in Table 1.

Hydroxypropyl cellulose was dissolved in mixture solution of water and Propylene glycol. Meloxicam was separately dissolved in ethanol containing transdermal enhancers. Afterwards, the both components were mixed and the hydrogels were stored in air-tight containers at room temperature prior to use.

III. *In-Vitro Skin Penetration Experiments*

The extent and rate of skin permeation of meloxicam from gel formulations were determined using a modified glass diffusion cell fitted with excised rat skin or human skin from breast reduction operations. 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 in the receptor compartment. The donor cell was filled with 3 g of 3% meloxicam gel and occluded by paraffin. The receptor compartment was filled with 20 ml of pH 7.4 phosphate buffer containing 20% ethanol and 20% PG and at 37 ±0.5°C controlled by thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm^2. Approximately 0.5 mL of the receptor 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. Each data represents the average of three determinations.

IV. *HPLC Analysis of Meloxicam*

The quantitative determination of meloxicam was performed by high performance liquid chromatography (HPLC). A HPLC equipped with a Hitachi model L-7100 pump, a Hitachi model L-4000H detector, a Spark Holland basic Marathon autosampler and Merck Lichrocart® C18 column (55×4 mm I.D., particle size 3 μm) was performed. Piroxicam was prepared as the internal standard. The mobile phase was a mixture of 0.05M di-ammonium phosphate (adjust 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 μg/mL (signal-to-noise > 4). The concentration range of the meloxicam in plasma was found to have linearity from 0.05 to 2 mg/mL (r^2 = 0.999). The coefficient variation of accuracy and precision for intraday and interday assay was 9.77 and 6.88%, respectively.

Table 1. Level, composition and responses of meloxicam model formulations.

Run	Azone		SDS		Menthol		Flux	Q48 hr	Lag time
	Code	%	Code	%	Code	%	μg/cm^2 hr	μg/cm^2	hr
Control		0		0		0	0.57 ± 0.11	30 ± 3	3.19 ± 0.99
R1	1	0	3	4	2	1	12.01 ± 1.54	949 ± 360	8.28 ± 0.33
R2	2	2	6	10	4	3	308.95 ± 36.79	13283 ± 1796	2.66 ± 1.45
R3	3	4	2	2	6	5	226.71 ± 0.56	10621 ± 1032	-0.67 ± 0.70
R4	4	6	5	8	1	0	304.50 ± 6.74	10319 ± 302	2.77 ± 0.84
R5	5	8	1	0	3	2	157.29 ± 6.73	7289 ± 585	4.64 ± 1.20
R6	6	10	4	6	5	4	250.29 ± 13.43	11416 ± 631	0.34 ± 0.27
R7	7	12	7	12	7	6	339.81 ± 18.24	14928 ± 941	-1.45 ± 0.85

Meloxicam and HPC were fixed at 3% and 3% respectively.
Lag time: the intercept on the time axis of the steady state flux calculated by linear regression.
Q48: Cumulative amount at 48 hr.

Table 2. The independent variables (factors) and dependent variables (responses) of the uniform design.

Run	Azone %	SDS %	Menthol %	EF	EQ48h (μg/cm^2)	Lag Time (LT) (hr)
R1	0	4	1	16.33	31.49	2.60
R2	2	10	3	539.65	440.67	0.83
R3	4	2	5	395.99	352.38	-0.21
R4	6	8	0	531.88	431.93	0.87
R5	8	0	2	274.74	241.82	1.45
R6	10	6	4	437.19	378.75	0.11
R7	12	12	6	593.54	495.27	-0.45

The effect of enhancer on flux (EF) and cumulative amount at 48 hr (EQ_{48h}) was calculated according to the relationship: $Value_{gel}$ / $Value_{control}$. The control formulation gel was without any enhancers.

V. *Data Analysis*

The cumulative amount of the drug penetration through rat skin was plotted as a function of time and a linear regression analysis was used to calculate the flux and lag time of the drug. The effect of enhancer was calculated according to the relationship: $Value_{gel}$ / $Value_{control}$. The control formulation gel was without any enhancers. The effect of enhancer on flux (EF), cumulative amount at 48 hr (EQ) and lag time (EL) were selected as the response variables. Statistical analysis including stepwise regression and response surface analysis were conducted using SAS software. Experimental designs resulted in a quadratic polynomial equation: $y=b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$, in which y is the dependent variable (response), b0 is a constant representing the mean of the dependent variable obtained in each experiment; x_1, x_2, and x_3 are the independent variables; x_1x_2, x_1x_2, and x_2x_3 are the interaction terms; x_1^2, x_2^2, and $x_3^2$are the quadratic term, and b_1, b_2…are the coefficients. This expression gives an insight into the effect of the different independent variables (response). A positive sign of coefficient indicates a synergistic effect, whereas a negative term indicates an antagonistic effect upon the response. Large coefficient means the causal factor has potent influence on the response. Afterward, two- and three-dimensional contour diagrams visualizing the simultaneous effect of the causal factors on the response at each time pint were established. Response surface analysis using the contour diagrams was utilized to select the formulation variables required to produce the desired values.

RESULTS AND DISCUSSION

I. *In-Vitro Skin Penetration Experiments*

The permeation profiles of these meloxicam model formulations through excised rat skin are shown in Figure 1. The rat skin penetration profile of meloxicam exhibited a zero-order permeation at a constant penetration rate

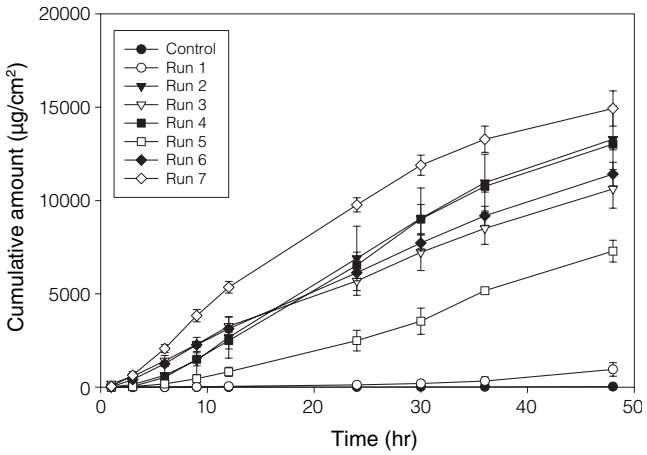

Figure 1. *In vitro* penetration-time profile of meloxicam model formulations and control gel (without penetration enhancers) through rat skin. (n = 3)

($R^2 > 0.8967$). The result of percutaneous rate (flux), cumulative amount at 48 hr (Q_{48h}) and lag time (LT) of these formulations are shown in Table 1. The flux, Q48h and lag time were from 12.01 μg/cm^2/hr to 339.81 μg/cm^2/hr, from 949 μg/cm^2 to 14928 μg/cm^2 and from 0 hr to 8.28 hr, respectively. The wide variation indicated the different enhancers combination could resulted in different enhancement effect on drug penetration thorough skin. The effect of enhancer on flux (EF), cumulative amount at 48 hr (EQ_{48h}), and LT were used as response variables (Table 2). The response surface models were calculated with SAS software. The model described the EF, EQ_{48h} and LT can be written as:

$EF = -133.72+118.01x_1+29.39x_2+30.11x_3-9.36x_1^2+0.93x_2^2-0.23x_3^2$

$EQ_{48h} = -108.96+93.59x_1+25.35x2+31.54x_3-0.10x_2x_3-2.49x_1^2+0.21x_2^2$

$LT = 9.56-1.64x_1-0.49x_2-0.32x_3-0.26x_1x_3+0.10x_2x_3+0.19x_1^2$

The x_1, x_2 and x_3 presented the concentration of azone, SDS and menthol, respectively. The two-dimensional response surfaces illustrating the simultaneous effect of the causal factors on each response variable are represented in Figure

Journal of Food and Drug Analysis, Vol. 14, No. 3, 2006

2. In order to easily evaluate the effect of main effect (x_1, x_2 and x_3), the regression coefficients were standardized by dividing the value of dependent standard deviation/ independent standard deviation by the statistical parameters for each response variables (Table 3).

For EF and EQ_{48h}, the positive standardized coefficients of main effect (x_1, x_2 and x_3) indicated that the penetration rate increased with increase in enhancers concentration. The rank order of standardized coefficient was $x_1 > x_2 > x_3$, indicated that azone had the most potential influence on the penetration absorption of meloxicam, followed by SDS and menthol.

In the case of LT, the negative standardized coefficients of main effect (x_1, x_2 and x_3) indicated that the lag time dropped by the increase in enhancers concentration. In agreement with above EF and EQ_{48h} finding, the effect of enhancers on LT was in the order of Azone > SDS > menthol.

From the EF equation, the two terms containing x_1 ($+118.01x_1–9.36x_1^2$) showed that the EF increased with increasing concentration of azone to 6.3% but decreased with further increasing concentration to 12%. Similarly the two x_1 terms ($+93.59x_1–2.49x_1^2$) in EQ_{48h} equation showed that the EQ_{48h} was increased with increasing concentration of azone to 6.4% and then found to decrease with further increasing concentration to 12%. In the case

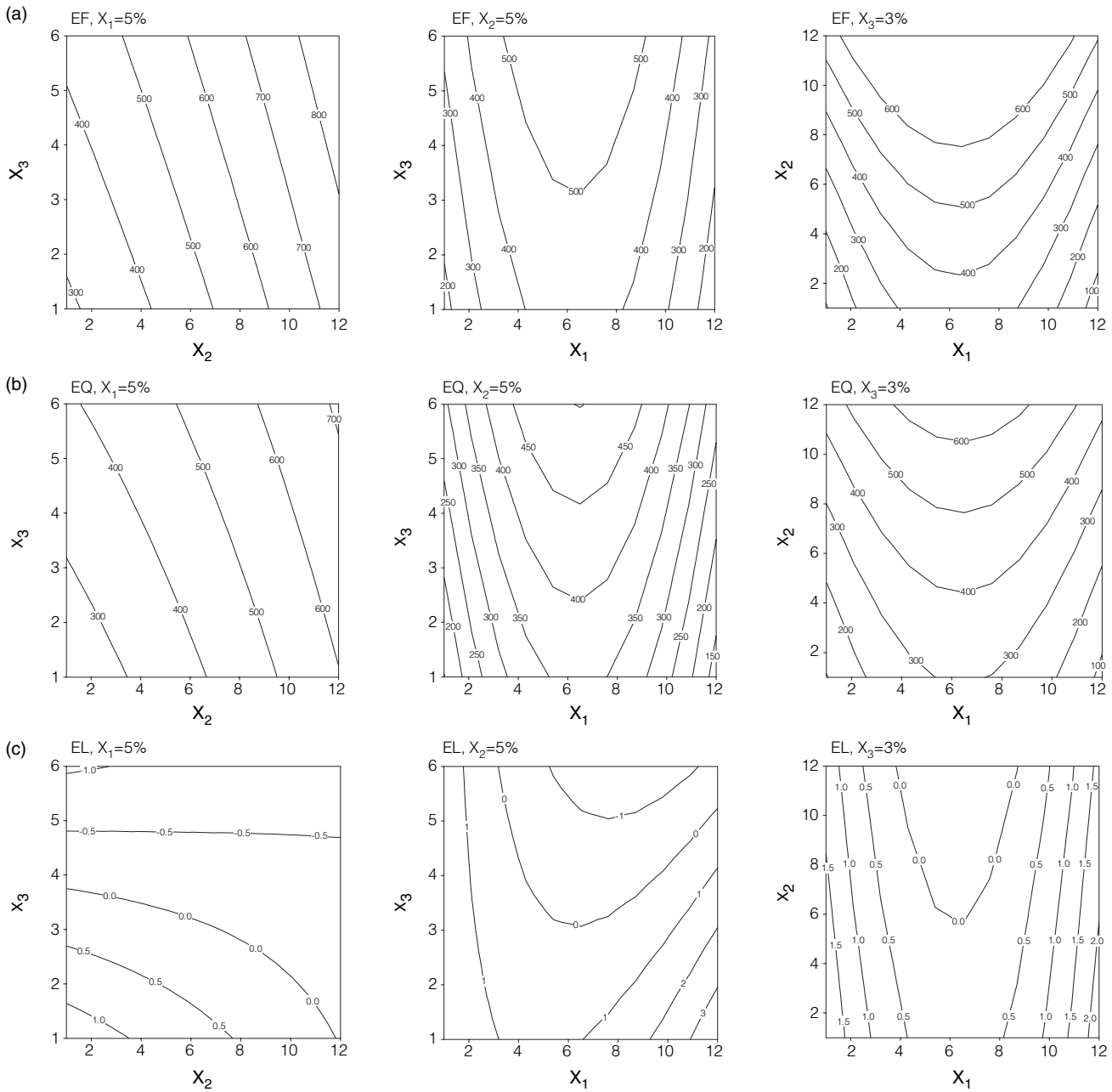

Figure 2. Two-dimensional contour diagrams illustrating the effect of penetration enhancers on the penetration absorption of meloxicam gel on moderate level. (a) EF (b) EQ (c) EL

of EL, there were three terms containing x_1 ($-1.64x_1 - 0.26x_1 x_3 + 0.19x_1^2$). If x_3 was fixed at moderate level (x_3 = 3%), LT decreased with an increase concentration of azone to 6.4% and increased with further increasing concentration to 12%. Therefore, in order to obtain higher penetration rate and shorter lag time of meloxicam gel, the optimal addition concentration of azone should be 4% to 6%. From Figure 2, it was shown that as the concentration of SDS increased from 0% to 12%, the EF and EQ_{48h} increased and LT decreased. The anionic surfactants cause greater enhancement and extensive damage to skin than nonionic surfactants and non-surfactant[13], since the lower addition amount of SDS was better for reducing the skin irritation. In comparison of the effect of menthol on EF, EQ_{48h} and LT, the result showed that the menthol had more influence on LT than on EF and EQ_{48h} (Table3). The result was similar to the report of Morimoto *et al.*[16] which indicated that menthol has the advantage of a shorter lag time and so used in many topical formulations containing nonsterodial anti-inflammatory drugs to increase the pharmaceutical effects.

Table 3. The standardized parameters of each response variable determined by multiple regression analysis.

Regression coefficient	EF	$EQ4_{8h}$	LT
$b_1(x_1)$	118.01	93.59	-1.64
$b_2(x_2)$	29.39	25.35	-0.49
$b_3(x_3)$	30.11	31.54	-0.32
$b_{11}(x_1x_1)$	9.36	-2.49	0.19
$b_{22}(x_2x_2)$	0.93	0.21	---
$b_{33}(x_3x_3)$	-0.23	---	---
$b_{12}(x_1x_2)$	---	---	---
$b_{13}(x_1x_3)$	---	---	-0.26
$b_{23}(x_2x_3)$	---	-0.10	0.10
R-square	0.9786	0.9712	0.9408
Adj R-square	0.9687	0.9579	0.9134
Standard deviation	8.30	9.26	43.57
F-value	98.87	72.98	34.41
Probability	0.0001	0.0001	0.0001

In order to validate the predictive ability of the hypothesized model for each response, additional gels were prepared (Table 4) and *in vitro* release studies were conducted in order to calculate the experimental EF, EQ_{48h} and LT. The predicted error of EF and EQ_{48h} were below 15% (Table 4). Although the predicted LT values was lower than experimental values, but there was a trend which the experimental values increased with increase in predicted values. The result showed that the response surface methodology (RSM) and multiple response optimization utilizing a polynomial equation can be successfully used to design a meloxicam hydrogel.

In addition, the required flux of meloxicam gel to maintain a therapeutic concentration was about 400 μg/hr in accordance with the previously studies[4] which reported that after oral administration 7.5 mg meloxicam in human, the C_{max} and Clearance are 0.88 mg/L and 0.42-0.48 L/hr, respectively. The flux of predicted formulations P4 through human skin was about 25.78 $\mu g/hr/cm^2$. The required minimum administration area of these formulations to reach therapeutic blood concentration was about 16 cm^2, which was within the appreciate range of application, indicting that it was possibly developed for the transdermal drug delivery system. However, clinical *in vivo* studies were needed to validate the feasibility of meloxicam transdermal delivery in humans.

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Table 4. The composition and responses of predicted formulations.

	P1	P2	P3	P4
$(EF)_{Predicted}$	503.60	509.32	459.88	478.89
$(EF)_{Experimental}$	444.45 ± 12.63	481.53 ± 43.04	459.74 ± 39.08	408.68 ± 22.46
Predict Error (%)	11.74	5.46	0.03	14.66
$(EQ_{48h})_{Predicted}$	431.87	431.20	415.87	418.09
$(EQ_{48h})_{Experimental}$	380.33 ± 32.88	416.02 ± 38.36	392.84 ± 35.87	355.57 ± 19.57
Predict Error (%)	11.93	3.52	5.54	14.95
$(Lag\ time)_{Predicted}$	-0.71	-0.82	-0.03	-2.81
$(Lag\ time)_{Experimental}$	0.22 ± 0.19	0.38 ± 0.28	1.05 ± 0.11	0.21 ± 0.03

1. The effect of enhancer on flux (EF) and cumulative amount at 48 hr (EQ_{48h}) was calculated according to the relationship: $Value_{gel}$ / $Value_{control}$. The control formulation gel was without any enhancers.
2. Predict Error = $\left|$Predicted value – Experimental value$\right|$ / Predicted value × 100%
3. P1: gels consisted of 4% azone, 5% SDS and 5% menthol.
4. P2: gels consisted of 5% azone, 5% SDS and 4% menthol.
5. P3: gels consisted of 6% azone, 5% SDS and 3% menthol.
6. P4: gels consisted of 6% azone, 3% SDS and 5% menthol.

Journal of Food and Drug Analysis, Vol. 14, No. 3, 2006

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