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In vitro evaluation of nimodipine permeation through human epidermis using response surface methodology

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Abstract

An optimization technique (response surface method) was used in order to investigate the effect of the combination of two enhancers, namely caprylic acid and cineol on nimodipine's permeation through human cadaver epidermis. Using this quadratic model it was found that at 24 h the increase of the permeation of nimodipine it was mainly due to the effect of caprylic acid. On the contrary, it was shown that at 48 and 72 h the combination of the two enhancers contributed to the increase of the permeation. The greater $Q_{gel}/Q_{control}$ values, at all time intervals (24, 48 and 72 h), were obtained when the concentration of cineol and caprylic acid range from 3.0 to 5.0% (v/v) and 8.0 to 9.5% (v/v), respectively.

Keywords: Nimodipine; Transdermal; Response surface methodology

1. Introduction

Nimodipine is a 1,4-dihydropyridine calcium antagonist, with a strong antispastic action on cerebral arteries (Haws and Heistad, 1984; Gaad et al., 1985) used in the treatment of senile dementia and in the prophylaxis of the vascular hemicrania (Manhold, 1985; Freedman and Waters, 1987). In view of its physicochemical and pharmacokinetic characteristics (Kirsch et al., 1984; Takata and Kato, 1986; Kumana et al., 1993), it seems that there is potential for investigating the ability of nimodipine to permeate human epidermis.

Various studies have demonstrated that the transdermal pathway may be a suitable alternative to the oral route in the administration of drugs with systemic activity. The potential advantages associated with transdermal drug delivery are well documented (Berba and Benakar, 1990; Wester and Maibach, 1992) and include avoidance of first-pass effect, administration of lower doses, potentially decreased side effects, constant plasma levels and improved patients compliance.

In the development of a transdermal drug delivery system the skin penetration enhancement of the drug is a key factor because of the barrier properties of the stratum corneum to drug permeation (Walters and Hadgraft, 1993).

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In a previous study, the effect of the type of enhancer on the transdermal permeation of nimodipine was investigated (Giannakou et al., 1998). It was found that myristyl alcohol, caprylic acid, L-menthol and oleic acid gave greater permeation rates at 24 h, while higher permeation rates at 48 and 72 h were achieved only when cineol was used.

In the present study the effect of the combination of caprylic acid and cineol on nimodipine's permeation through human cadaver epidermis was investigated, in order to achieve higher and sustained permeation rates at all time intervals (24, 48 and 72 h), using an optimization technique (response surface methodology).

2. Materials and methods

2.1. Materials

The materials used were: Nimodipine (Batch: 540043, Help Ltd, Greece), hydroxypropyl cellulose or HPC (Aqualon, USA), glycerin BP/USP (Unichema, Germany), *n*-octanoic acid (caprylic acid) (Sigma Chemical Co., USA), cineol (eucalyptol, Sigma Chemical Co., USA), sodium azide (Sigma Chemical Co., USA), methanol (Lab Scan, HPLC grade, Ireland), acetonitrile (Lab Scan, HPLC grade, far UV, Ireland) and absolute ethanol (Riedel-de Haen, USA).

All the materials were used as received without further purification.

2.2. Methods

2.2.1. Preparation of nimodipine formulations

The preparation of the formulations were as follows: 190 mg of nimodipine were dissolved in 10 ml of a solvent system (EtOH/H_2O/Glycerin: 60/30/10, v/v) and the amounts of the appropriate enhancers were then added. These solutions were gelled with 2.0% (w/v) hydroxypropyl cellulose (HPC).

2.2.2. Quantitative determination of nimodipine in the gels

For the quantitative analysis, accurately weighed samples of gels were placed in volumetric flasks, diluted to a certain volume with mobile phase and stirred for 30 min. The samples were then filtered through a 0.45 μ filter (Type 589, Schleicher and Schuel, Germany) and analyzed by a modified HPLC method (Yoshida et al., 1990). All experiments were performed in triplicate ($n = 3$).

2.2.3. HPLC method

The HPLC system consisted of a high pressure pump (P1000, Spectra Physics, USA), an autosampler (AS 1000, Spectra Physics, USA), equipped with a Spherisorb ODS2-S10 column (25 cm × 4.6 mm, Phase Separation, UK), a variable wavelength detector (Spectra 100 UV–Vis detector, Thermo Separation Products, USA) set at 240 nm and an integrator (SP 4400 integrator, Chromjet, Thermo Separation Products, USA). The mobile phase consisted of methanol, acetonitrile and water (MeOH/AcN/H2O: 40/40/20 v/v) and was pumped through the column at a flow rate of 1.5 ml/min. The injection volume was 10 μl.

2.2.4. In vitro release studies

The in vitro release studies were carried out using modified Franz diffusion cells, with 6.3 ml volume and 0.636 cm^2 diffusion surface area. In the donor compartment 100 μl of nimodipine gel were applied. The receptor fluid was a solution of sodium azide 0.02% (w/v) and Tween 40, 1% (v/v). Human epidermal membrane, taken from full thickness cadaver skin by heat separation technique (Kligman and Christophers, 1963), was mounted between the donor and receptor compartments. The studies were carried out at 32 ± 0.5 °C. Four cells were used for each formulation. Samples were taken at predetermined time intervals (6, 12, 24, 36, 48, 72 h) and analyzed by HPLC. In every series of experiments a gel without enhancer was used as a control formulation.

2.2.5. Experimental design

Before building an experimental design (Wehrle et al., 1993), all variables-independent and dependent—and the experimental field must be defined precisely.

Table 1
Factors (independent variables) and levels of the response surface design

Factors	High level (%)	Middle level (%)	Low level (%)
Cineol (X)	10	5	0
Caprylic acid (Y)	10	5	0

In the design used in this study, two factors at three levels each were used. Each enhancer represents a factor or independent variable (Table 1). In every formulation the concentrations of caprylic acid and cineol were varied. All other parameters were kept constant.

The experimental field of the design must not be too large, leading to non-realistic experiments and not too small, far from an optimal region. In this study, the lower level for the concentration of the enhancers was 0%, v/v, which represents the absence of the enhancer the middle level is 5%, v/v and the high level was 10%, v/v (Table 1).

In vitro skin flux experiments were carried out and the amount of nimodipine permeated per unit area of epidermal membrane (Q: μg/cm^2) after 24,

Table 2
Effect of the combination of enhancers on the permeation of nimodipine from gels (HPC 2% w/v, nimodipine 1.9% w/v, EtOH/H2O/Glycerin: 60/30/10, v/v)

Enhancers in formulations (v/v)	Q (μg/cm^2) (24 h)	SD ($n=4$)	Q (μg/cm^2) (48 h)	SD ($n=4$)	Q (μg/cm^2) (72 h)	SD ($n=4$)
Control	10.0	0.7	12.3	0.6	12.8	0.7
5% Cineol	25.0	5.2	33.6	5.2	38.6	4.8
10% Cineol	12.5	4.5	19.4	5.8	25.7	7.0
5% Caprylic acid	31.6	4.0	33.8	3.4	35.4	3.9
5% Cineol and 5% caprylic acid	26.2	3.5	39.3	3.9	46.6	4.5
10% Cineol and 5% caprylic acid	14.2	0.5	22.6	0.6	31.5	0.2
10% Caprylic acid	33.9	6.6	38.3	8.0	40.5	8.5
5% Cineol and 10% caprylic acid	20.9	2.3	35.4	3.9	45.9	6.8
10% Cineol and 10% caprylic acid	14.3	1.6	22.2	3.3	32.9	4.7

Table 3
Factors (independent variables) and responses (dependent variables) of the response surface design

Independent variables		Dependent variables (responses)		
Cineol, % (v/v)	Caprylic acid, % (v/v)	$Q_{gel}/Q_{control}$		
X	Y	$Z_1 \pm$ SD (24 h)	$Z_2 \pm$ SD (48 h)	$Z_3 \pm$ SD (72 h)
0	0	1.0 ± 0.01	1.0 ± 0.01	1.0 ± 0.01
5	0	2.5 ± 0.15	2.7 ± 0.09	3.0 ± 0.08
10	0	1.3 ± 0.11	1.6 ± 0.11	2.0 ± 0.16
0	5	3.2 ± 0.11	2.8 ± 0.05	2.8 ± 0.06
5	5	2.6 ± 0.08	3.2 ± 0.06	3.6 ± 0.08
10	5	1.4 ± 0.01	1.8 ± 0.01	2.5 ± 0.01
0	10	3.4 ± 0.25	3.1 ± 0.23	3.1 ± 0.24
5	10	2.1 ± 0.04	2.9 ± 0.06	3.6 ± 0.16
10	10	1.4 ± 0.08	1.8 ± 0.04	2.6 ± 0.08

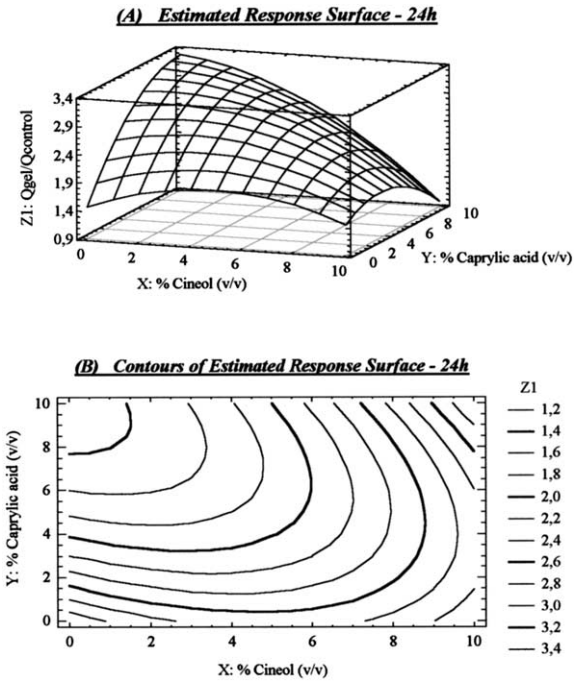

Fig. 1. Estimated response surface (A) and contour plot (B), illustrating the relationship between the concentration of cineol (% v/v) and caprylic acid (% v/v) and the permeation rate of nimodipine at 24 h (Z_1), expressed as $Q_{gel}/Q_{control}$ values.

48 and 72 h was measured. The response or dependent variables Z_1, Z_2 and Z_3, were then calculated according to the relationship: $Q_{gel}/Q_{control}$, for 24, 48 and 72 h, respectively. This relationship is used for normalizing the data, gathered from several transdermal permeation studies using different skin donors.

To describe the response surface curvature a 3^2 experimental design was built and the quadratic model can be calculated by Eq. (1):

$$Z = A_1 + A_2X + A_3Y + A_4X^2 + A_5Y^2 + A_6XY \quad (1)$$

where Z: response variable; X: % v/v, concentration of cineol; Y: % v/v, concentration of caprylic acid; A_1: constant and A_2, A_3, A_4, A_5, A_6: regression coefficients.

The constant and the regression coefficients were calculated using a commercial software package (Mini10XTRA, Minitab Incorporated). The polynomial equations from this optimization technique can be used in order to predict the permeation of nimodipine, using combinations of cineol and caprylic acid.

In order to check the validity of the model, analysis of variance was used. F-ratios and correlation coefficients were the criteria for validation.

In addition to the statistical validation, a series of experiments were conducted in order to challenge the reliability of the response surface model, by comparing the predicted values for Z_1, Z_2 and Z_3 with the experimental data.

3. Results and discussion

Based on Table 1, nine formulations were prepared using combinations of the two factors at their levels. After conducting the in vitro release studies the amount of nimodipine permeated per unit area of epidermal membrane (Q: μg/cm^2) was measured at 24, 48 and 72 h. The results are shown in Table 2. Based on these data, the effect of enhancer is calculated according to the relationship: $Q_{gel}/Q_{control}$. As control formulation the gel with both the enhancers at their low level (0% v/v) is used. The normalized data, used as response variables, are depicted in Table 3. The response surface models were then calculated by multiple regression analysis (Minitab 10XTRA, Minitab Incorporated).

The constant, the regression coefficients and the statistical parameters for each response variable, were as follows:

$$Z_1 = 1.458 + 0.178X + 0.365Y - 0.018X^2 - 0.018Y^2 - 0.023XY \quad (R^2 = 0.79;\ s = 0.6;\ F_{5,3} = 2.4;\ P = 0.252) \quad (2)$$

$$Z_2 = 1.269 + 0.405X + 0.345Y - 0.037X^2 - 0.017Y^2 - 0.019XY \quad (R^2 = 0.91;\ s = 0.4;\ F_{5,3} = 6.1;\ P = 0.088) \quad (3)$$

$$Z_2 = 1.236 + 0.508X + 0.352Y - 0.043X^2 - 0.017Y^2 - 0.015XY$$

$$(R_2 = 0.95;\ s = 0.3;\ F_{5,3} = 10.4;\ P = 0.042) \quad (4)$$

The response surfaces and the contour plots for Z_1, Z_2 and Z_3 are shown in Figs. 1 and 2 and Fig. 3, respectively.

From Fig. 1 it is shown that as the concentration of caprylic acid increases from 0 to 10% (v/v) the ratio $Q_{gel}/Q_{control}$ also increases, mainly when cineol is kept at low concentration (lower of 5% v/v). On the contrary, the increase of cineol concentration does not result to an increase of the ratio $Q_{gel}/Q_{control}$. This finding can be explained by analyzing the data of Eq. (2). From this equation it is obvious that the regression coefficient of X (0.178, $P_X = 0.43$) is smaller than the regression coefficient of Y (0.365, $P_Y = 0.16$). Therefore, the main effect on the permeation of nimodipine at 24 h is mainly due to the existence of caprylic acid in the formulation.

These results are in agreement with the results of a previously reported study (Giannakou et al., 1998), which has shown that the main effect of caprylic acid is observed at 24 h.

On the contrary, at 48 and 72 h the ratio $Q_{gel}/Q_{control}$ increases not only when the concentration of caprylic acid increases, but also when the concentration of cineol increases up to 5%

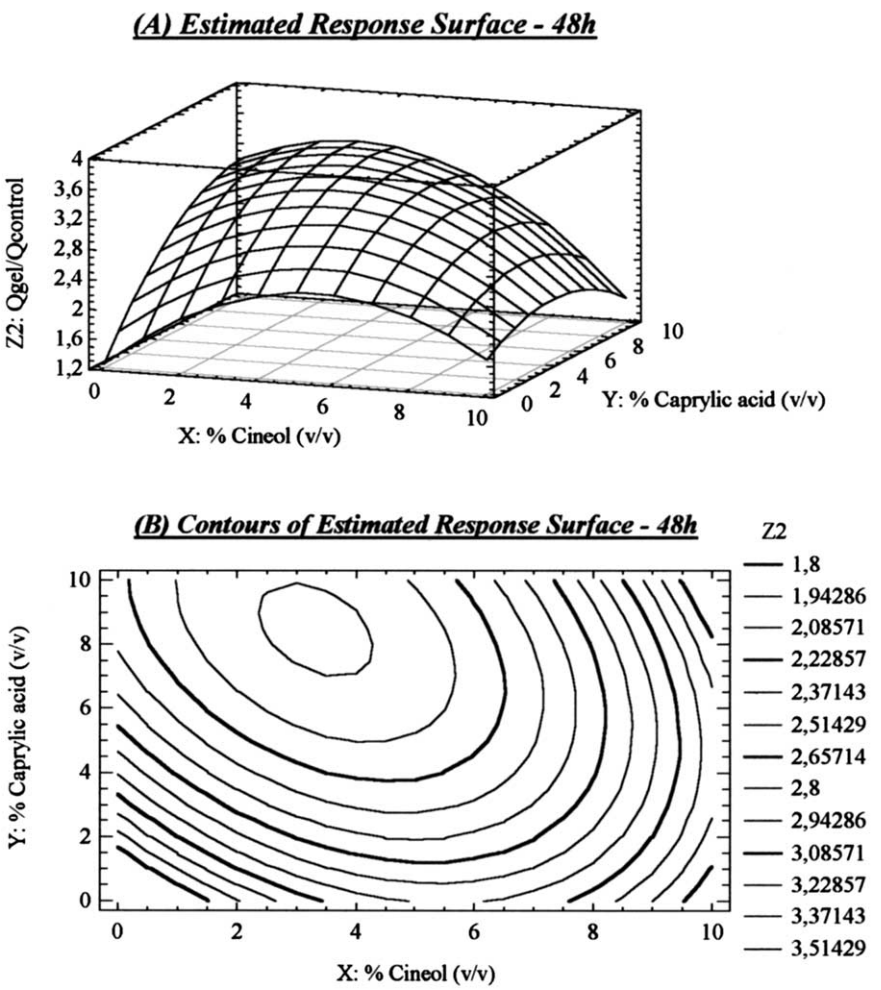

Fig. 2. Estimated response surface (A) and contour plot (B), illustrating the relationship between the concentration of cineol (% v/v) and caprylic acid (% v/v) and the permeation rate of nimodipine at 48 h (Z_2), expressed as $Q_{gel}/Q_{control}$ values.

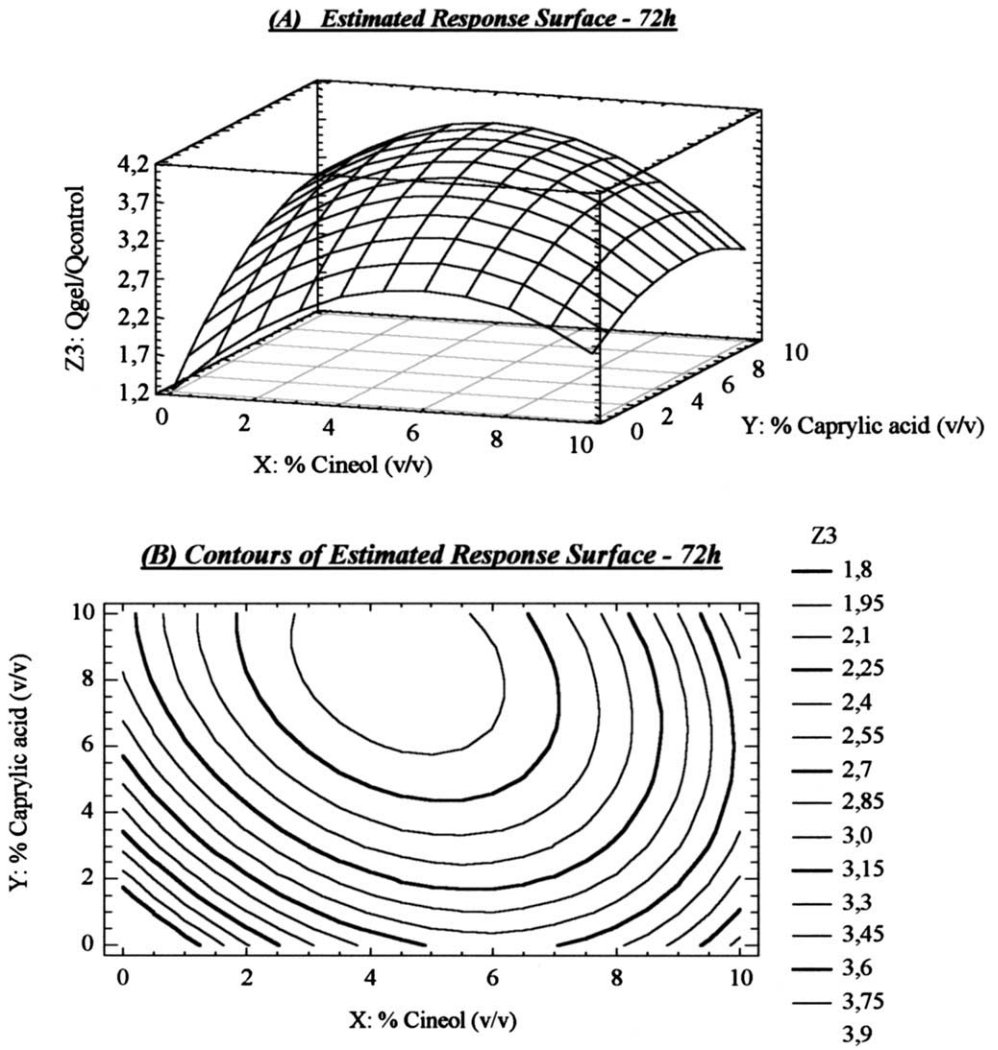

Fig. 3. Estimated response surface (A) and contour plot (B), illustrating the relationship between the concentration of cineol (% v/v) and caprylic acid (% v/v) and the permeation rate of nimodipine at 72 h (Z_3), expressed as $Q_{gel}/Q_{control}$ values.

(v/v), as it is shown from Fig. 2 and Fig. 3. From Eq. (3) and Eq. (4) it is obvious that the regression coefficient of X becomes greater at 48 h (0.405, $P_X = 0.04$) and 72 h (0.508, $P_X = 0.01$). At the same time intervals the regression coefficient of Y is practically not changing (0.345, $P_Y = 0.06$ and 0.352, $P_Y = 0.03$ at 48 and 72 h, respectively). Therefore, cineol sustains and increases the transdermal permeation of nimodipine after 24 h either alone or in combination with caprylic acid.

The ability of cineol to increase the transdermal permeation has been reported also for other compounds, such as chloropromazine and haloperidol (Almirall et al., 1996) and insulin (Ogiso et al., 1996).

The greater $Q_{gel}/Q_{control}$ values, at all time intervals (24, 48 and 72 h), were obtained when the concentration of cineol and caprylic acid range from 3.0 to 5.0% (v/v) and 8.0 to 9.5% (v/v), respectively.

A series of experiments were conducted in order to challenge the reliability of the response surface

Table 4
Experimental data ($Q = \mu g/cm^2$) from several gels used to evaluate the reliability of the response surface method

Gels with enhancers (v/v)	Experimental data					
	Q ($\mu g/cm^2$) (24 h)	SD ($n = 4$)	Q ($\mu g/cm^2$) (48 h)	SD ($n = 4$)	Q ($\mu g/cm^2$) (72 h)	SD ($n = 4$)
3.0% Cineol and 3.0% caprylic acid	19.21	1.53	40.56	3.40	53.00	4.54
7.5% Cineol and 2.5% caprylic acid	15.98	0.81	35.92	0.32	50.81	0.86
3.5% Cineol and 8.5% caprylic acid	22.05	0.61	48.32	1.54	66.54	1.89
7.0% Cineol and 7.0% caprylic acid	18.00	0.37	40.56	1.46	57.40	2.27
Control	7.32	0.05	14.32	0.11	17.74	0.09

Gels consisted of VHL, HPC 2% w/v, nimodipine 1.9% w/v and enhancers.

Table 5
Predicted, experimental data of $Q_{gel}/Q_{control}$ and statistical criteria used to evaluate the reliability of the response surface method

Z_1—24 h					
Gels with enhancers (v/v)	Predicted $Q_{gel}/Q_{control}$	Experimental $Q_{gel}/Q_{control} \pm$ SD	P/E	$\lvert E-P \rvert$	$(\lvert P-E \rvert)/P \times 100$
3.0% Cineol and 3.0% caprylic acid	2.56	2.62 ± 0.10	0.98	0.06	2.34
7.5% Cineol and 2.5% caprylic acid	2.15	2.18 ± 0.08	0.99	0.03	1.40
3.5% Cineol and 8.5% caprylic acid	2.98	3.01 ± 0.16	0.99	0.03	1.01
7.0% Cineol and 7.0% caprylic acid	2.39	2.46 ± 0.12	0.97	0.07	2.93
Z_2—48 h					
3.0% Cineol and 3.0% caprylic acid	2.87	2.83 ± 0.11	1.01	0.04	1.39
7.5% Cineol and 2.5% caprylic acid	2.66	2.51 ± 0.12	1.06	0.15	5.64
3.5% Cineol and 8.5% caprylic acid	3.41	3.37 ± 0.06	1.01	0.04	1.17
7.0% Cineol and 7.0% caprylic acid	2.99	2.83 ± 0.10	1.06	0.16	5.35
Z_3—72 h					
3.0% Cineol and 3.0% caprylic acid	3.15	2.99 ± 0.10	1.05	0.16	5.08
7.5% Cineol and 2.5% caprylic acid	3.14	2.86 ± 0.15	1.10	0.28	8.92
3.5% Cineol and 8.5% caprylic acid	3.85	3.75 ± 0.03	1.03	0.10	2.60
7.0% Cineol and 7.0% caprylic acid	3.60	3.26 ± 0.11	1.10	0.34	9.44

model. Additional gels were prepared (Table 4) and in vitro release studies were conducted in order to calculate the experimental $Q_{gel}/Q_{control}$ values for Z_1, Z_2 and Z_3. The experimental data was then compared with the predicted values derived from Eqs. (2)–(4).

In order to validate the agreement between the predicted values and the experimental data the following statistical criteria were examined (Wayne, 1991):

(a) the ratio PREDICTEDvalue/EXPERIMENTALvalue, which should be close to one ($P/E \cong 1$)
(b) the standard error, which should be close to zero ($|E - P| \cong 0$)
(c) the % relative standard deviation, which should be lower than 15%.
(d) The results presented in Table 5 indicate an acceptable agreement between the predicted values and the experimental data.

4. Conclusions

With the aid of an experimental design technique the effect of the combination of two enhancers, namely caprylic acid and cineol, on the transdermal permeation of nimodipine was studied. Using the response surface methodology the sustained action of cineol on nimodipine's skin permeability, after 24 h, was confirmed either alone or in combination with caprylic acid.

Our results also indicate that the polynomial equations from this optimization technique could be used in order to predict the transdermal permeation of nimodipine from formulations with certain combinations of caprylic acid and cineol. The greater $Q_{gel}/Q_{control}$ values, at all time intervals (24, 48 and 72 h), were obtained when the concentration of cineol and caprylic acid range from 3.0 to 5.0% (v/v) and 8.0 to 9.5% (v/v), respectively.

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