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Development and in vitro evaluation of nitrendipine transdermal formulations using experimental design techniques

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Abstract

In the present study nitrendipine was incorporated into gels and its efficacy to permeate human epidermis was examined in vitro. A preliminary study was carried out in order to estimate the effect of the type of enhancer, the concentration of enhancer and the concentration of gelling agent on the flux of nitrendipine, using a 2^3 factorial design. The type of enhancer and the concentration of enhancer were further evaluated as they were found to be important for nitrendipine flux, while the concentration of the gelling agent was kept at its optimum level in all experiments. In order to increase further the flux of nitrendipine, the combination of two enhancers, glycerol monooleate (GMO) and *N*-methyl-2-pyrrolidone (NMP), which act via different mechanisms, at three concentration levels was examined, using the response surface method. The results indicate that higher flux values were obtained when NMP was greater than 4.5% w/w and GMO between 5.0 and 9.5% w/w, in the vehicle.

Keywords: Nitrendipine; Transdermal; Experimental design

1. Introduction

Nitrendipine, a 1,4-dihydropyridine derivative calcium entry blocker, is a potent peripheral vasodilator which effectively reduces blood pressure, when given at doses of 5–20 mg/day. After a single, 20 mg oral dose of nitrendipine, peak plasma concentrations (which vary widely from 5 to 40 μg/l) are achieved within 1–2 h (Goa and Sorkin, 1987). Nitrendipine has a terminal elimination half-life of between 10 and 22 h, is 98% bound to plasma proteins and is reported to be well absorbed following oral administration, but undergoes extensive first-pass metabolism; the absolute oral bioavailability is reported to range from 10 to 20% (Kann et al., 1984; Lasseter et al., 1984).

In view of the physicochemical and pharmacokinetic characteristics of nitrendipine (e.g., small oral dose, low molecular weight, lipid solubility and an extensive first-pass effect), it seems that there is potential for investigating the ability of nitrendipine to permeate human epidermis. The importance of the vehicle in percutaneous absorption is well documented (Loth, 1991). It is also well known that the flux of drugs through the stratum corneum can be increased with transder-

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mal penetration enhancers, due to their ability to change the structure of lipophilic and/or keratinized domains in stratum corneum (Santus and Baker, 1991). In the present study, a certain vehicle is used for nitrendipine while different enhancers, at various concentrations, were examined, using experimental design techniques (factorial design and response surface methods), in order to find the most appropriate formulations for the transdermal application of nitrendipine.

2. Materials and methods

2.1. Materials

The materials used were nitrendipine (batch NTR-189, Lusochimica, Italy), glycerol caprylate/caprate and PEG-8 caprylate/caprate (Labrasol, Gattefosse S.A., France), ethoxydiglycol (Transcutol, Gattefosse S.A., France), polysorbate 20 (Tween 20 (T20), ICI, Italy), polysorbate 80 (Tween 80 (T80), ICI, Italy), dodecyl alcohol (lauryl alcohol (L.A.), Sigma Chemical Co., USA), oleic acid (O.A.) (E. Merck, Germany), glycerol monooleate or GMO (Eastman Kodak, USA), L-menthol (L-M) (A. Maschemeijer Jr, The Netherlands), *N*-methyl-2-pyrrolidone (NMP) (GAF, USA), sodium azide (Sigma Chemical Co., USA), hydroxypropyl cellulose (HPC) (Aqualon, USA), methanol and tetrahydrofuran (Lab Scan, HPLC grade, Ireland), absolute ethanol (Riedel-de Haen, USA) and glycerol BP/USP (Unichema, Germany). All the materials were used as received without further purification.

2.2. Methods

2.2.1. Solubility studies

Solubility studies were conducted by adding excess amounts of nitrendipine into sealed vials containing 10 g of vehicle. The vials were placed on a rotating disk for 24 h (25°C). Samples were filtered through a 0.45 μm filter (type 589, Schleicher and Schuel, Germany) and the concentration of nitrendipine in the filtrate was determined according to a modified high-pressure liquid chromatography (HPLC) method (Aronoff and Sloan, 1985). All experiments were performed in triplicate ($n = 3$).

2.2.2. 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 238 nm and an integrator (SP 4400 integrator, Chromjet, Thermo Separation Products, USA). The mobile phase consisted of methanol, tetrahydrofuran and water (MeOH/THF/H_2O, 45:20:35 v/v) and was pumped through the column at a flow rate of 1.5 ml/min. The injection volume was 10 μl.

2.2.3. Preparation of standard solutions

The mother solution was prepared by dissolving a known amount of nitrendipine in absolute ethanol. From this solution five standard stock solutions were prepared with appropriate dilutions, in mobile phase. Calibration curves, constructed on the basis of peak height vs concentration, were found to be linear over the concentration range studied (correlation coefficients ranged from 0.996 to 0.999).

2.2.4. Preparation of nitrendipine gels

Nitrendipine (140 mg) was dissolved in an EtOH/H_2O/glycerine 60:30:10 w/w solvent system. A measured amount of the appropriate enhancer was then added and the solutions were gelled with 2.0% (w/w) HPC.

2.2.5. Quantitative determination of nitrendipine in the gels

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 μm filter and injected into the HPLC system. All experiments were performed in triplicate ($n = 3$). The results of the quantitative analysis were acceptable (between 95 and 105% of the theoretical value).

Table 1
Levels, main effects and interactions of the factors on solubility of nitrendipine using a 2^3 factorial design

Factors	High (+)	Low (−)
A: H_2O/EtOH/glycerine	30:60:10	45:45:10
B: type of enhancer	Labrasol	lauryl alcohol
C: concentration of enhancer	5%	2%

	Factors			Effects on solubility	*F*
	A	*B*	*C*		
Main effects					
A: H_2O/EtOH/glycerine	+	−	−	4.5842	3 362 456
B: type of enhancer	−	+	−	−0.5775	53 361
C: concentration of enhancer	−	−	+	0.7573	91 748
Interactions					
AB	+	+	−	0.0283	128
AC	+	−	+	0.1230	2 421
BC	−	+	+	−0.1888	5 700
ABC	+	+	+	−0.0025	1

In vitro release studies

In vitro release studies were carried out using modified Franz diffusion cells of 6.3 ml volume and 0.636 cm^2 diffusion surface area. In the donor compartment 100 μl of nitrendipine gel were applied, while the receptor fluid was a solution of sodium azide 0.02% (w/v). Human epidermal membrane, taken from full-thickness cadaver skin using a 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 (3, 6, 12, 24, 36, 48, 60 and 72 h) and analyzed by HPLC. In every series of experiments, a gel without enhancer was used as a control formulation.

3. Results and discussion

3.1. Selection of vehicle

The selection of the vehicle was based on a factorial design of three factors at two levels 2^3 (Armstrong and James, 1990). The aim of the factorial design was to determine the factor with the most important effect on the solubility of nitrendipine. The factors and levels, high (+) and low (−), are listed in Table 1. The solubility of nitrendipine in the vehicles was measured as a response.

Based on the data from Table 1, factor *A* was found to be the most important as far as the solubility of the drug is concerned. Therefore, the vehicle EtOH/H_2O/glycerine 60:30:10 w/w (high level of factor *A*, Table 1) was selected for further evaluation.

Table 2
Solubility of nitrendipine (mg/ml) in VHL after adding various enhancers (5% w/w)

Solutions	Solubility (mg/ml) $\pm$ SD ($n = 3$)
VHL/oleic acid	16.331 ± 0.047
VHL/NMP	16.329 ± 0.381
VHL/L-menthol	17.396 ± 0.184
VHL/lauryl alcohol	16.467 ± 0.096
VHL/GMO	16.051 ± 0.059
VHL/Labrasol	16.324 ± 0.025
VHL/Tween 20	15.538 ± 0.045
VHL/Tween 80	16.498 ± 0.101
VHL/Transcutol	17.030 ± 0.070
VHL	13.692 ± 0.126

Table 3
Levels, main effects and interactions of the factors on the flux of nitrendipine using a 2^3 factorial design

Factor	High (+)	Low (−)
A: type of enhancer	Labrasol	lauryl alcohol
B: concentration of enhancer	5%	2%
C: concentration of HPC	2%	1%

	Factors			Effects on flux	*F*
	A	*B*	*C*		
Main effects					
A: type of enhancer	+	−	−	−0.360	12.96
B: concentration of enhancer	−	+	−	−0.265	7.02
C: concentration of HPC	−	−	+	0.300	9.00
Interactions					
AB	+	+	−	0.100	1.00
AC	+	−	+	−0.145	2.10
BC	−	+	+	−0.160	2.56
ABC	+	+	+	0.105	1.10

3.2. Solubility studies

Solubility studies were conducted to determine the maximum concentration of nitrendipine dissolved in the vehicle EtOH/H_2O/glycerine 60:30:10 w/w (VHL) after adding various enhancers. Based on the solubility data shown in Table 2, the concentration of nitrendipine in each formulation was chosen to be 14 mg/ml.

3.3. In vitro release studies

A factorial design 2^3 (Table 3) was used in order to determine the effects of the type of enhancer, concentration of enhancer and concentration of gelling agent on nitrendipine flux. The response measured was the flux, i.e., the amount of nitrendipine permeated/unit area of epidermal membrane per unit time ($\mu g/cm^2$ h). The results are shown in Table 3.

It is obvious from Table 3 that all three factors affect the flux of nitrendipine, while their interactions were found to be not significant. The effect of the type of enhancer was further evaluated by incorporating different enhancers into VHL at 5% w/w concentration (Table 2). Their effect on the flux of nitrendipine at 24, 48 and 72 h is shown in Table 4. The concentration of HPC was kept at a high level in all formulations (2% w/w).

Table 4
Effect of the type of enhancer on the flux of nitrendipine (HPC 2% w/w, nitrendipine 1.4% w/w, enhancer 5% w/w)

	Formulations	Flux at 24 h ($\mu g/cm^2$ h)	SD ($n = 4$)	Flux at 48 (h $\mu g/cm^2$ h)	SD ($n = 4$)	Flux at 72 (h $\mu g/cm^2$ h)	SD ($n = 4$)
Skin 1	VHL/O.A./HPC/NIT	3.04	0.31	1.98	0.27	1.80	0.26
	VHL/GMO/HPC/NIT	2.62	0.11	2.08	0.11	2.01	0.06
	VHL/NMP/HPC/NIT	1.88	0.14	1.37	0.09	1.13	0.10
	VHL/L.A./HPC/NIT	2.24	0.16	1.82	0.14	1.65	0.17
	VHL/Labrasol/HPC/NIT	1.04	0.16	0.77	0.14	0.66	0.12
	VHL/Transcutol/HPC/NIT	1.24	0.34	0.85	0.24	0.72	0.22
	VHL/HPC/NIT (control)	0.87	0.19	0.56	0.11	0.48	0.11
Skin 2	VHL/L-M/HPC/NIT	0.55	0.10	0.95	0.35	0.73	0.27
	VHL/T20/HPC/NIT	0.52	0.14	0.32	0.04	0.26	0.05
	VHL/T80/HPC/NIT	0.68	0.40	0.41	0.20	0.32	0.18
	VHL/HPC/NIT (control)	0.34	0.15	0.43	0.18	0.38	0.14

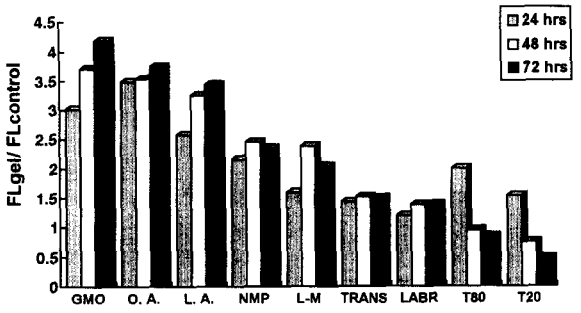

Fig. 1. Effect of several enhancers on the flux of nitrendipine (normalized flux data vs control formulation).

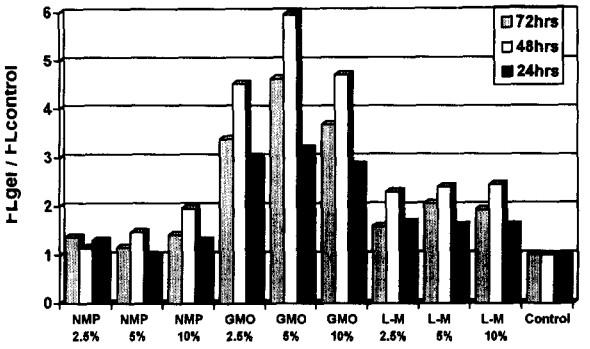

Fig. 2. Effect of the concentration of enhancers on the flux of nitrendipine (normalized flux data vs control formulation).

From the data listed in Table 4, the effect of enhancer is calculated according to the relationship: $FL_{gel}/FL_{control}$ (FL, flux). This relationship is used for normalizing the data, gathered from several flux studies using different skin donors. The normalized data are depicted in Fig. 1.

The effects of the various enhancers on the flux of nitrendipine follow the order: GMO > oleic acid > lauryl alcohol > NMP > L-menthol > Transcutol > Labrasol > Tween 80 > Tween 20, as demonstrated in Fig. 1. The first three enhancers act via the same mechanism, as they cause temporary and reversible disruption of the ordered lamellar structure of the bilayers in the stratum corneum, leading to increased fluidization of the intercellular lipid medium (Friend et al., 1989; Kai et al., 1990). NMP appears to partition preferentially into the keratin region (Ghosh and Banga, 1993), while L-menthol has the same mechanism of action as the first three enhancers. From all the enhancers tested, GMO, NMP and L-menthol were selected for further evaluation, since they act through different mechanisms and/or belong to different chemical categories. Transcutol, Labrasol, Tween 20 and Tween 80 were not further studied, since they gave flux values closer to the control formulation. The selected enhancers (GMO, NMP, L-menthol) were incorporated into VHL, at three concentration levels (2.5, 5 and 10% w/w). Flux studies were then conducted and the results are presented in Table 5.

The effect of the concentration of enhancers on nitrendipine flux is depicted in Fig. 2 as the

Table 5
Flux values for nitrendipine gels, using enhancers at three concentration levels (gels consisted of VHL, HPC 2% w/w, nitrendipine 1.40% w/w and enhancer)

Gels with enhancers (w/w)	Flux ($\mu g/cm^2$ h) AT 24 h	SD ($n = 4$)	Flux ($\mu g/cm^2$ h) at 48 h	SD ($n = 4$)	Flux ($\mu g/cm^2$ h) at 72 h	SD ($n = 4$)
NMP 2.5%	0.61	0.06	0.41	0.03	0.33	0.02
NMP 5%	0.45	0.07	0.31	0.04	0.28	0.04
NMP 10%	0.61	0.14	0.41	0.07	0.34	0.05
GMO 2.5%	1.40	0.05	0.95	0.24	0.81	0.18
GMO 5%	1.49	0.13	1.25	0.31	1.11	0.23
GMO 10%	1.33	0.07	0.99	0.07	0.88	0.06
L-M 2.5%	0.78	0.23	0.48	0.13	0.38	0.10
L-M 5%	0.75	0.10	0.50	0.11	0.49	0.05
L-M 10%	0.75	0.07	0.51	0.08	0.46	0.07
Control	0.47	0.04	0.21	0.10	0.24	0.02

Table 6
Factors (independent variables), levels and responses (dependent variables) of the response surface design

Factors	High	Middle	Low
GMO (X)	10%	5%	0%
NMP (Y)	10%	5%	0%

Independent variables		Dependent variables (responses)		
% GMO (w/w) (X)	% NMP (w/w) (Y)	$FL_{gel}/FL_{control}$ at 24 h (Z_1)	$FL_{gel}/FL_{control}$ at 48 h (Z_2)	$FL_{gel}/FL_{control}$ at 72 h (Z_3)
0	0	1.0	1.0	1.0
5	0	3.3	4.0	3.7
10	0	2.6	2.8	2.9
0	5	1.6	2.0	1.8
5	5	4.8	4.2	3.4
10	5	3.7	3.8	3.1
0	10	1.2	1.2	1.1
5	10	4.7	4.1	3.2
10	10	4.5	4.4	3.6

ratio $FL_{gel}/FL_{control}$. It is obvious that, by varying the concentration of NMP and L-menthol, the flux of nitrendipine was not significantly affected. In contrast, the increase in GMO concentration from 2.5 to 5% (w/w) increases nitrendipine flux, while further increase, from 5 to 10% (w/w) results in a decrease in the flux.

In order to achieve higher flux values for nitrendipine, a response surface method (Stetsko, 1986; Bolton, 1990) was applied and the effect of the combination of two enhancers (NMP, GMO) on the flux of nitrendipine was examined. The percentages (w/w) of GMO (X) and NMP (Y) at three levels (Table 6) were the independent vari-

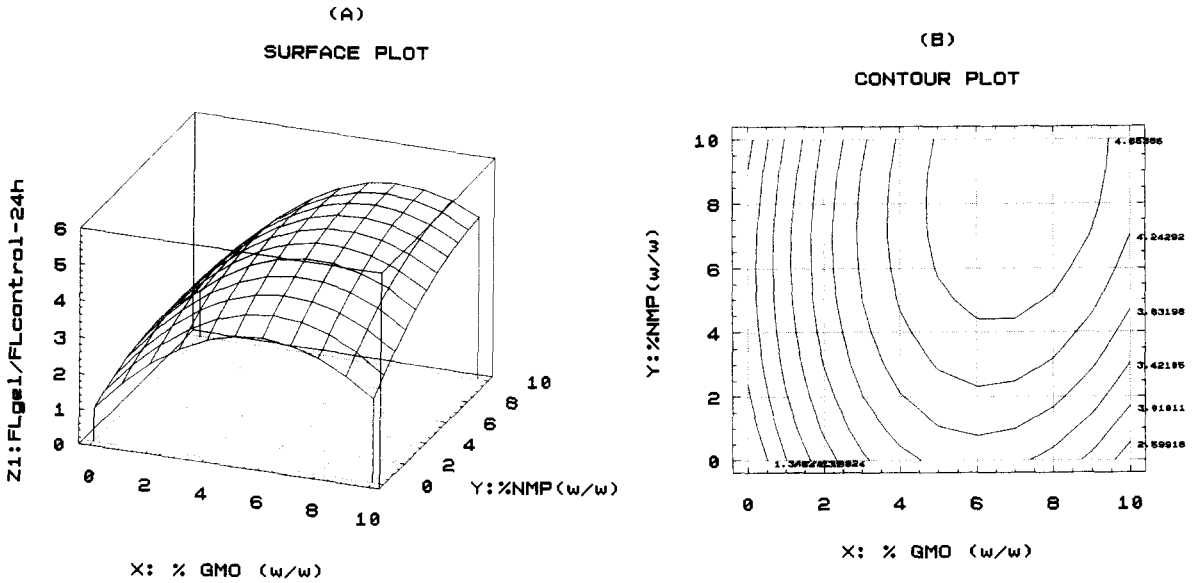

Fig. 3. Response surface (A) and contour (B) plots for $FL_{gel}/FL_{control}$ values of nitrendipine at 24 h (Z_1).

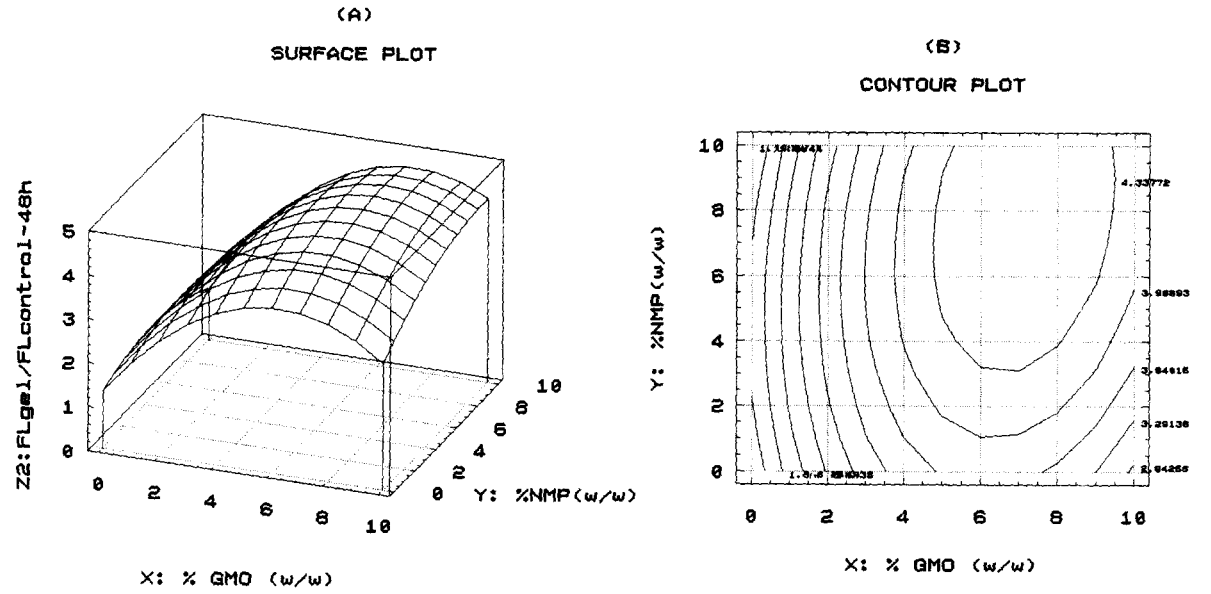

Fig. 4. Response surface (A) and contour (B) plots for $FL_{gel}/FL_{control}$ control values of nitrendipine at 48 h (Z_2).

ables while $FL_{gel}/FL_{control}$ values at 24 h (Z_1), 48 h (Z_2) and 72 h (Z_3) were the responses (Table 6). GMO was selected as a factor due to its significant effect on nitrendipine flux, while NMP was chosen due to its different mechanism of action. Based on Table 6, nine formulations were prepared using combinations of the two factors at their levels.

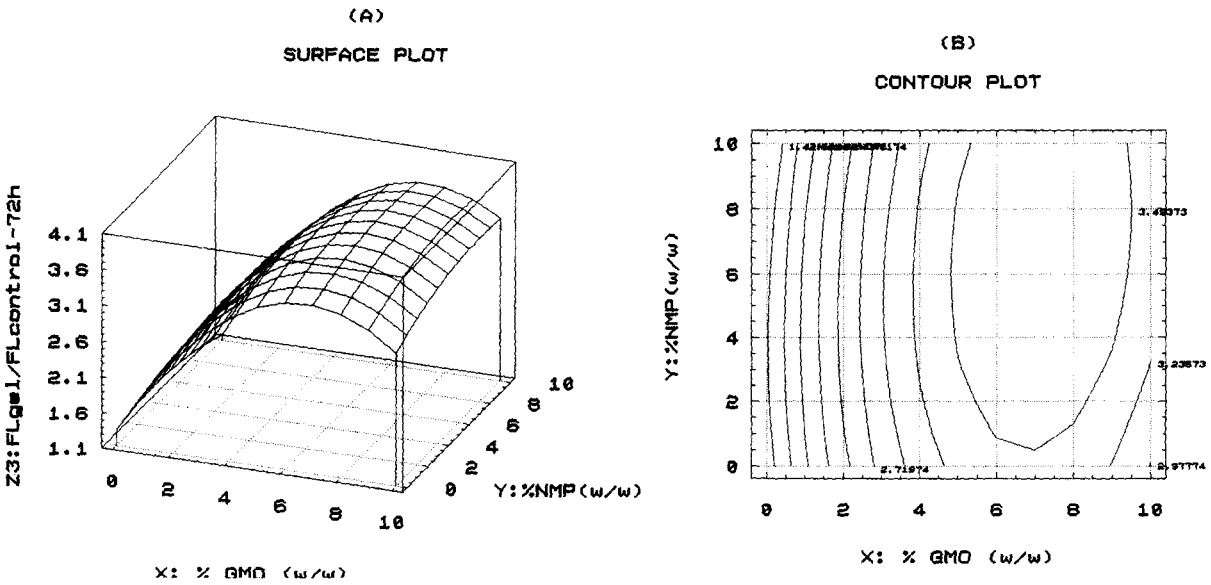

Fig. 5. Response surface (A) and contour (B) plots for $FL_{gel}/FL_{control}$ control values of nitrendipine at 72 h (Z_3).

Table 7
Predicted and experimental data of $FL_{gel}/FL_{control}$, for several formulations used to evaluate the reliability of the response surface method

	Formulations with enhancers (w/w)	$FL_{gel}/FL_{control}$	
		Predicted	Experimental
Z_1 24 h	2.5% NMP	1.382	1.173
	2.5% GMO	2.700	2.692
	10% NMP	1.232	0.975
	6.5% GMO	3.568	2.541
	6.5% GMO and 10% NMP	5.015	2.639
Z_2 48 h	2.5% NMP	1.563	1.171
	2.5% GMO	2.880	2.714
	10% NMP	1.198	1.085
	6.5% GMO	3.766	2.294
	6.5% GMO and 10% NMP	4.576	3.411
Z_3 72 h	2.5% NMP	1.400	1.100
	2.5% GMO	2.606	2.700
	10% NMP	1.172	1.113
	6.5% GMO	3.453	2.381
	6.5% GMO and 10% NMP	3.662	3.786

Formulations consisted of VHL, HPC 2% w/w, nitrendipine 1.40% w/w and enhancer).

The response surface models were calculated by multiple regression analysis. The polynomial equations obtained and their statistical parameters were as follows:

$$Z_1 = 0.95 + 0.88X + 0.22Y - 0.07X^2 - 0.02Y^2 + 0.02XY$$
$$(R^2 = 0.97; s = 0.26; F_{5,3} = 55.13; p < 0.004)$$

$$Z_2 = 1.30 + 0.79X + 0.14Y - 0.06X^2 - 0.02Y^2 + 0.01XY$$
$$(R^2 = 0.92; s = 0.39; F_{5,3} = 18.50; p < 0.01)$$

$$Z_3 = 1.32 + 0.63X + 0.05Y - 0.05X^2 - 0.01Y^2 + 0.01XY$$
$$(R^2 = 0.84; s = 0.41; F_{5,3} = 9.60; p < 0.04)$$

The response surfaces and the contour plots for Z_1, Z_2 and Z_3 are shown in Figs. 3–5, respectively.

From Figs. 3–5 it is shown that, as the concentration of GMO increases up to 5% (w/w), the ratio $FL_{gel}/FL_{control}$ also increases. A smaller increase of the ratio also occurs as the concentration of NMP increases from 0 to 10% (w/w). It is also evident that the combination of GMO and NMP results in higher $FL_{gel}/FL_{control}$ values. Thus, the maximum $FL_{gel}/FL_{control}$ values, at all time intervals (24, 48 and 72 h), are obtained when NMP $\geq$ 4.5% and 5.0% $\leq$ GMO $\leq$ 9.5% (w/w) are used.

A series of experiments were conducted in order to evaluate the reliability of the response surface model, by comparing the predicted values for Z_1, Z_2 and Z_3 with the experimental data. The data from Table 7 indicate an acceptable agreement between the predicted values and the experimental data.

4. Conclusions

From the experiments conducted using a 2^3 factorial design, it was demonstrated that the type and the concentration of enhancer and the concentration of gelling agent affect the flux of nitrendipine, while their interactions were found to be not significant.

The enhancers examined in this study are arranged in diminishing efficacy, as far as nitrendipine flux is concerned, as follows: GMO > oleic acid > lauryl alcohol > NMP > L-menthol > Transcutol > Labrasol > Tween 80 > Tween 20.

The increase in L-menthol concentration in gels from 2.5 to 10% (w/w) does not affect ni-

trendipine flux, while the increase in NMP concentration to 10% (w/w) results in a small increase in flux; moreover, the increase in GMO concentration to 5% (w/w) increases the flux of nitrendipine. The greater flux value for nitrendipine was obtained at all time intervals (24, 48 and 72 h) when 5% (w/w) GMO was used.

When the GMO and NMP combination was used, even higher $FL_{gel}/FL_{control}$ values were achieved, as shown with the application of the response surface method. The polynomial equations from this optimization technique can be used in order to predict the flux values of nitrendipine, from the VHL vehicle using certain combinations of GMO and NMP. Thus, the maximum flux values were obtained when NMP $\geq$ 4.5% and 5.0% $\leq$ GMO $\leq$ 9.5% (w/w) were used.

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