Applying response surface methodology to optimize nimesulide permeation from topical formulation

Yasser Shahzad[1]*, Urooj Afreen[2], Syed Nisar Hussain Shah[2], Talib Hussain[1]

[1]Division of Pharmacy and Pharmaceutical Science, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, United Kingdom

[2]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan

*Correspondence: Division of Pharmacy and Pharmaceutical Science, School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH, United Kingdom

Email: y.shahzad@hud.ac.uk

Abstract

Nimesulide is a non-steroidal anti-inflammatory drug that acts through selective inhibition of COX-2 enzyme. Poor bioavailability of this drug may leads to local toxicity at the site of aggregation and hinders reaching desired therapeutic effects. This study aimed at formulating and optimizing topically applied lotions of nimesulide using an experimental design approach, namely response surface methodology. The formulated lotions were evaluated for pH, viscosity, spreadability, homogeneity and *in vitro* permeation studies through rabbit skin using Franz diffusion cells. Data were fitted to linear, quadratic and cubic models and best fit model was selected to investigate the influence of permeation enhancers, namely propylene glycol and polyethylene glycol on percutaneous absorption of nimesulide from lotion formulations. The best fit quadratic model explained that the enhancer combination at equal levels significantly increased the flux and permeability coefficient. The model was validated by comparing the permeation profile of optimized formulations' predicted and experimental response values, thus, endorsing the prognostic ability of response surface methodology.

Keywords: Nimesulide, response surface methodology, lotion, permeation, permeability coefficient, rabbit skin

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs for symptomatically alleviating pain and swelling associated with conditions such as arthritis, toothache, dysmenorrhea and other musculoskeletal disorders. NSAIDs act by inhibiting inflammatory mediators, namely cyclo-oxygenase (COX) enzymes, which are responsible for producing prostaglandins (1). The COX-1 isoform is implicated in homeostasis while COX-2 is particularly associated with inflammatory reactions (2, 3). Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) is the first marketed NSAID that act through selective inhibition of COX-2 (1, 4, 5). Structurally, nimesulide contains sulfoanilide moiety which makes it a weekly acidic drug (pK_a=6.5) as shown in Figure 1.

Figure 1

This drug presents a very low aqueous solubility (0.01 mg/mL), an octanol-water partition coefficient (logP) of 2.60 and low bioavailability, therefore, it has been classified as Class II drug according to Biopharmaceutics Classification System (BCS) (6, 7). Poor bioavailability of this drug may leads to local toxicity at the site of aggregation and hinders reaching desired therapeutic effects (7). Taking into consideration the physicochemical properties and poor bioavailability of nimesulide, it can be a good candidate for transdermal drug delivery as an alternative to oral route of its delivery.

Transdermal drug delivery has gained significant attention in recent years. It provides several advantages over oral route including patient compliance, avoidance of gastrointestinal untoward effects and maintains a steady state plasma concentration (8). Transdermal drug delivery facilitates the passage of therapeutic quantities of drug through the skin into the general circulation, thus bypassing the hepatic first pass effect (9). Research has been carried out to overcome the barrier properties of the stratum corneum (SC) of the skin using physical and chemical methods. The physical enhancement techniques currently in use, for example iontophoresis and sonophoresis, require complex equipment (9, 10). Alternatively, chemical enhancement techniques involved use of chemical compound known as permeation enhancers which temporarily lower the impermeability of SC, thus facilitate the drug to pass through the skin. Commonly used permeation enhancers are alcohols with long carbon chains, cyclic monoterpenes, surfactants, pyrrolidones, propylene glycol, isopropyl myristate and dimethyl sulfoxide (11-13).

In the development of transdermal formulations, it is essential to design an optimized formulation that has appropriate percutaneous absorption. In recent years a computer optimization technique based on response surface methodology (RSM) has been widely practiced (14-19). The methodology encompasses utilization of polynomial equations and mapping of the responses over the experimental domain to quantify the influence of formulation variables on the drug permeation and assist predicting the optimal formulation. It reduces the number of experimental runs necessary to establish a mathematical trend in the experimental design allowing for the determination of the optimum level. Reducing the number of experiments by optimizing a formulation during development of a drug delivery device may also lead to significant reductions in production costs (20).

The present study aimed at formulating and optimizing the permeation of nimesulide from its topical lotion formulation using experimental design. All the formulated lotions were subjected to physical characterization and *in vitro* permeation across rabbit skin. RSM was employed to assess the influence of formulation variables on the percutaneous absorption of nimesulide. Data were assessed to predict the optimized formulation to validate the model.

Experimental

Materials

Nimesulide 99.9 % purity (Merck, Germany), propylene glycol (Merck, Germany), polyethylene glycol (PEG-400) (Fluka, Germany), isopropyl alcohol (IPA) (Merck, Germany), methanol-HPLC grade 99% (Merck, Germany), Tween-20 (Merck, Germany), potassium di-hydrogen phosphate (Fluka, Germany), sodium chloride (Merck, Germany), potassium chloride (Sigma-Aldrich, UK), di-Sodium hydrogen phosphate (Fluka, Germany), vacuum Grease (Dow Corning, USA), carbopol-940 (Merck, Germany) and sodium hydroxide (Shama Laboratory chemical works, Pakistan) were used as received.

High-performance liquid chromatography (HPLC) analysis

Quantitative analysis of nimesulide was performed as described previously (21) using a Waters HPLC system (Elstree, UK) equipped with a 600E pump, a 484 UV-visible detector, an autosampler and a C18 Nucleosil® 5 µm column of 150 mm length and 4.5 mm internal diameter (Alltech Associates, Deerfield IL). The mobile phase consisted of acetonitrile–methanol–15 mM potassium di-hydrogen phosphate buffer, pH 7.3 (30:5:65 v/v). Mobile phase was filtered through 0.45 µm filter and degassed using ultrasonic bath for 30 minutes

prior to use. The flow rate was adjusted to 1 mL/min and UV detector was set at 393nm wavelength. The HPLC analysis was performed at ambient temperature.

Solubility Studies

The solubility of nimesulide was measured in various solvents: distilled water, phosphate buffered saline (PBS; pH 7.2), methanol, mixture of PBS-methanol (1:1 v/v), propylene glycol (PG), and polyethylene glycol (PEG-400). An excess quantity of nimesulide was stirred with each of the solvent for 48 hours in thermostatic conditions (37± 2ºC). Samples withdrawn were filtered through 0.2 µm nylon filter (Fisher Scientific, UK) followed by dilution with appropriate solvent. The concentration of nimesulide was then determined in triplicate using HPLC.

Preparation of topical formulation

In order to optimize the formulation and valuation of the influence of formulation variables on nimesulide permeation, a central composite design (CCD) with $\alpha = 2$ was employed as per standard protocol. The factors, namely PG (X_1) and PEG (X_2) studied at 5 levels (-2, -1, 0, 1, 2) were selected based on the results of preliminary experiments. Preliminary experiments were conducted utilising PG and PEG combination at two concentrations, namely 5% and 40%. It was found that the combination of enhancers could enhance the permeation of nimesulide. Therefore, it was decided to optimize lotion formulations within the studied range. The central point (0, 0) was studied in quintuplicate. All other formulation and processing variables were kept invariant throughout the study as given in Table 1.

Table 1

Nimesulide hydro-alcoholic lotions (100 mL each) were prepared as per the CCD design as shown in Table 1. Essentially, 1 g nimesulide was dissolved in 20 mL of mixture of PBS-methanol (1:1 v/v) followed by the addition of PG and PEG according to the CCD design. It was stirred over a magnetic stirrer for 30 minutes until solution was homogenised. Isopropyl alcohol (20 mL) was taken in a separate flask and 0.2 g carbopol-940 was added to it with constant stirring. To this solution, 4 mL Tween-20 was added and it was stirred for another 30 minutes. Both solutions were then mixed over continuous stirring and final volume (100 mL) was achieved by adding mixture of PBS and methanol

(1:1 v/v). An enhancer (PG and PEG) free lotion was also prepared as control (L_C).

In vitro characterization

Each nimesulide containing lotion was subjected to tests in order to determine its pH, viscosity, spreadability, and homogeneity. Each of these studies was conducted in triplicate (n=3).

Lotion pH was measured with a digital pH meter (Mettler & Toledo, Giessen, Germany). Viscosity evaluations were conducted at room temperature (25 ± 2°C) using a Model RVTDV II Brookfield viscometer (Stoughton, MA). A C-50 spindle was employed, with a rotation rate of 220 rpm. The gap value was set to 0.3 mm.

The spreadability of each lotion was determined by the wooden block and glass slide method as detailed previously (22). Essentially, a 5mL volume of lotion was added to a dedicated pan and the time taken for a movable upper slide to separate completely from the fixed slides was noted. Spreadability was determined according to the formula:

$$S = \frac{M \times L}{t} \quad (1)$$

Where:

S = Spreadability

M = Weight/Volumes tide to upper slide

L = Length of glass slide

t = Time taken to separate the slide completely from each other

Each formulated lotion was evaluated for homogeneity by naked eye examination. This involved a subjective assessment of appearance including the presence of any aggregates.

Permeation studies

This study was conducted under the conditions that had been regulated and approved by the Animal Ethics Committee of Bahauddin Zakariya University, Pakistan. White New Zealand male rabbits weighing between 3-4 kg were used for the preparation of skin. The skin samples were excised from the abdomen region. Hairs were clipped short and adhering subcutaneous fat was removed carefully from the isolated full thickness skin. The skin was cut into samples that were just larger than the surface area of the Franz diffusion cells. To

remove extraneous debris and any leachable enzyme, the dermal side of the skin was kept in contact with a normal saline solution for 1 hour prior to start the diffusion experiments.

Permeation experiments were performed using Franz cells manufactured 'in house', exhibiting a diffusional area of 0.85cm^2 and a receptor cell volume of 4.5 mL. Subsequently, the test membrane was inserted as a barrier between the donor and receiver cells. Silicone grease was applied in order to create a good seal between the barrier and the two Franz compartments. To start each permeation experiment, 1 mL volume of each lotion formulation was deposited in the donor cell while receptor compartment was filled with PBS-methanol mixture (1:1 v/v). The diffusion cells were placed on a stirring bed (Variomag, US) immersed in a water bath at 37 ± 5°C to maintain a temperature of ~32°C at the membrane surface. At scheduled times, a 0.5 mL aliquot of receiver fluid was withdrawn and the receiver phase was replenished with 0.5 mL of fresh pre-thermostated PBS-methanol mixture. Withdrawn aliquots were assayed immediately by HPLC for nimesulide quantification. Sink conditions existed throughout. Since skin exhibits large sample-to-sample permeability differences (23), therefore, each experiment consisted of 5 replicate runs (n=5).

Data Analysis

According to Fick's second law of diffusion, the cumulative amount of drug (Q_t) appearing in the receptor solution in time t is expressed in Eq. 2:

$$Q_t = AKLC_0\left[\left(\frac{Dt}{L^2}\right)-\left(\frac{1}{6}\right)-\left(\frac{2}{\pi^2}\right)\Sigma\frac{(-1)^n}{n^2}\right]\times\exp\left(\frac{D^n 2\pi^2 t}{L^2}\right) \quad (2)$$

where A, is the effective diffusion area, C_0, represents the drug concentration which remains constant in the vehicle, D is the diffusion coefficient, L denotes the thickness of the membrane and K is the partition coefficient of the drug between membrane and vehicle. At steady state, it is expressed in Eq. 3:

$$\frac{Q_t}{A} = KLC_0\left[\left(\frac{Dt}{L^2}\right)-\left(\frac{1}{6}\right)\right] \quad (3)$$

The steady state flux (J) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area as a function of time, in the steady-state region where the

drug would pass by constant rate. The lag time was determined from the x-intercept of the slope at the steady state. The flux is expressed in Eq. 4;

$$J = \frac{C_0 KD}{L} = C_0 K_P \tag{4}$$

From this relation the permeability coefficient was calculated using Eq. 5;

$$K_P = \frac{J}{C_0} \tag{5}$$

The effectiveness of penetration enhancers (enhancement ratio, ER) was calculated from the ratio of nimesulide flux in the presence and absence of enhancers.

The analysis of responses, namely lag time (t_{lag}) and permeability coefficient (K_P) were performed using Minitab statistical software version 16. Linear, quadratic and cubic mathematical models were employed. The best fit model was selected based on the comparison of several parameters including the multiple correlation coefficients (R^2), adjusted multiple correlation coefficients (adjusted R^2), predicted residual sum of square (PRESS), and the lack of fit (p-value). Experimental design resulted in a quadratic polynomial equation which is expressed in Eq.6:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 - \beta_1^2 X_1^2 - \beta_2^2 X_2^2 \tag{6}$$

where Y is the dependent variable (response), β_0 is a constant representing the mean of the dependent variable obtained in each experiment; X_1 and X_2 are the independent variables; X_1X_2 are the interaction terms; X_1^2 and X_2^2 are the quadratic term and β_1, β_2…are the coefficients. This expression gives an insight into the effect of the different independent variables. 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. Afterwards contour and 3D-surface plots visualizing the simultaneous effect of the causal factors on the response were established (24).

The optimization and validation of experimental domain was performed by predicting optimum formulation using numerical optimizing provision of the Minitab software. The experimental response values and model predicted response values were compared and percentage predicted error was calculated. One-way ANOVA was applied to estimate the significance of the model ($p < 0.05$). All measured data are expressed as mean ± standard deviation (S.D.). Each measurement was executed in 5 replicates (n= 5).

Results and discussion

Solubility data

Table 2 illustrates the solubility of nimesulide in each of the studied solvents: distilled water, PBS, methanol, PBS-methanol mixture (1:1 % v/v), PG and PEG-400. Nimesulide is sparingly soluble in water (25, 26), for that reason, it was expected that solubility of nimesulide in water would be least as confirmed by the solubility studies. Solubility enhancement factor was calculated from the ratio of nimesulide solubility in water and different solvents. A 3.7-fold higher solubility was achieved in PBS (pH 7.2) which reflects the fact that the ionic form of nimesulide is more soluble than its neutral form. Solubility of nimesulide in PBS-methanol mixture (1:1 v/v) was 122-fold higher which might be due to the solvent polarity difference between two different solvent systems, namely water and PBS-methanol mixture. Maximum solubility of nimesulide was observed in PEG which was 6864-fold higher than that in water.

Table 2

In vitro characterization data

In vitro characterization includes pH, viscosity, spreadability and homogeneity. All the formulated lotions were appeared as clear, colourless and aggregate free homogeneous solutions upon preparation. All the lotions exhibited a pH value from 5.2 - 5.4 with no significant differences existing between each formulation (data not shown). However, variation in lotion viscosities with respect to PG and PEG content were observed among formulations. PEG has higher viscosity then PG, therefore, L3 and L9 showed highest viscosities owing to higher PEG levels in the formulations. It can be seen that the viscosities of all the enhancer containing lotions were significantly different ($p < 0.05$) form that of the control as confirmed by ANOVA (Table 3). Furthermore, spreadability data were inversely related to the viscosity of each of the nimesulide containing lotion formulation. With increasing viscosity, spreadability was decreased as shown in the Table 3. Statistical analysis showed significant difference ($p < 0.05$) between the formulations that were based on axial and central points of the CCD.

In vitro nimesulide permeation through rabbit skin

The *in vitro* permeation of nimesulide from its lotion formulation was studied using modified Franz cells across rabbit skin. Figure 2 illustrates the cumulative amount of drug permeated as a function of time from lotion formulations as per CCD. It can be seen from the Figure 2

that highest permeation was achieved for L_3 that contains equal amount of PG and PEG at high level. The steady state flux was calculated by a linear regression between cumulative amount permeated and time. The lag time (t_{lag}), which is directly related to the drug diffusivity, was calculated from the x-intercept of the cumulative amount of drug permeated as a function of time. The permeation parameters are listed in Table 3. The t_{lag} values for lotion formulations were ranged from 40.9 ± 2.45 min to 109.9 ± 11.8 min. The steady state flux (J) ranged from 118.6 ± 2.80 to 180.5 ± 15.9, permeability coefficient (K_P) ranged from 0.060 ± 0.001 to 0.091 ± 0.008, and enhancement ratio (ER) ranged from 2.22 to 3.39 for lotion formulations, which indicated that the permeation of nimesulide from its lotion formulation was significantly influenced by the proportion of the formulation variables, namely PG and PEG. Moreover, lag time, flux and permeability coefficient values for enhancer containing lotions were significantly different ($P < 0.05$) from that of the control (L_C).

There are various mechanisms associated with the permeation enhancement of drug by a permeation enhancer. They can increase the thermodynamic activity, they can increase skin/vehicle partition coefficient, they can increase the solubilizing power of the skin to the drug, or they can reversibly reduce the impermeability of skin (27). PG and PEG which are commonly used solvents in the pharmaceutical industries were employed in this study as permeation enhancers. Previous studies have postulated that PG may carry the drug through the barrier layer (28, 29) while other studies describe that PG as a hydrophilic material enters the keratin in the corneocyte but does not alter the lipid fluidity in hydrated tissue (30). More recently, it has been suggested that PG as a hydrophilic material with two hydroxyl group could replace water at the binding sites in the polar head group region and may act in a similar way to water (31). Kaushik and co-workers have reported that PEG has a retarding effect on permeation of diethyl-m-toluamide (mosquito repellent) through an anonymous mechanism compared to the several other permeation enhancers used in the study (32). As far as we could ascertain, there is no published report describing the effect of PG and PEG combination on percutaneous absorption of nimesulide.

Figure 2

Table 3

The influence of PG and PEG on the permeation of nimesulide from its lotion formulations was quantified by analysing the responses (t_{lag}, K_P) using RSM. The estimation of quantitative effects of the factor combination and their levels on responses was carried out by fitting data to linear, quadratic and cubic models. The best fit model was quadratic which could be represented as:

$$Y_1(t_{lag}) = 92.7 - 5.68(X_1) + 0.27(X_2) - 12.9(X_1X_2) - 0.88(X_1)^2 - 8.2(X_2)^2 \quad (7)$$

$$Y_2(K_P) = 7.68 + 0.38(X_1) + 0.13(X_2) + 0.70(X_1X_2) - 0.15(X_1)^2 - 0.16(X_2)^2 \quad (8)$$

The significance of formulation variables on nimesulide permeation was evaluated through multiple linear regression analysis using Minitab statistical software version 16. The comparative values of R^2, adjusted R^2, PRESS, lack of fit (*p*-value) are summarized in Table 4.

RSM data analysis

Analysis of RSM data revealed a significant model probability at *p*-value less than 0.05 and insignificant lack of fit at *p*-value greater than 0.05. This implies that the resultant could describe the relationship between factors and the responses. The main effects of X_1 and X_2 show the average result of changing one variable at a time from its low to higher level while interaction effects of X_1X_2, X_1^2 and X_2^2 represent the results when both factors were altered simultaneously. It was observed that responses were considerably influenced by the main effect and the interaction of the factors. More interestingly, interaction of factors (X_1X_2) influenced t_{lag}, K_P and *ER* relatively higher than the main effects indicating the PG and PEG combination was more suitable in enhancing permeation of nimesulide. The negative coefficients of X_1^2 and X_2^2 imply an unfavourable effect of the factors on the permeation of nimesulide. The R^2 values for Eq. 7 and 8 were found to be 0.583 and 0.776, respectively, indicating a reasonable correlation coefficient of the fitted model. The lag time values (Table 3) revealed significant difference between the formulations based on axial points of the CCD. The lag time is dependent on the rate at which drug diffuses through the skin, thus a higher drug diffusivity leads to reduction in lag time (29). The longest lag times were obtained for the formulations carrying medium or high levels of PG. This may be explained on the basis that the enhancing effect of PG is exerted by enhancing the drug partitioning into the stratum corneum. To do this, PG has to partition into the SC where it accumulates into the intercellular and protein regions of SC, thus changing its solubilizing power with subsequent

increased drug partitioning into the SC (30). The flux values for L_1, L_5, L_6, L_8 and L_{12} were found to be similar (Table 3) as these lotion formulations were based on central points of the CCD and have similar levels of PG and PEG. It should be noted that lotion formulation having equal amount of PG and PEG (L_3, formulations based on central points and L_{10}) showed a gradual increase in the permeation profile with respect to factor level with L_3 showing the highest flux and permeability coefficient as given in Table 3. The observed increase in the permeation profile of nimesulide, when enhancer combination was used in equal volume, could be attributed to the fact that PG dehydrates and desolvate the SC (31) and disrupts the lipid-protein complex with subsequent increased solubility of nimesulide in this membrane. Additionally, presence of PEG also contributed to increase in the solubility of nimesulide and may lose its retarding ability in the presence of PG which has disrupted the lipid-protein complex in the stratum corneum (32). The retarding effect of PEG is owing to its inability to hydrate the SC or its relative osmotic effect which tends to dehydrate the SC (33). Since PG tends to disturb the lipid-protein complex in the SC, it was assumed that PEG may not act as permeation retardant when SC is disrupted by co-enhancer. This resulted in an increased amount of solubilized nimesulide in the SC that creates a concentration gradient which facilitated the drug to permeate through the SC.

This was further analysed by constructing contour and 3-D surface plots (Figures 3 and 4) which are useful in visual explanation of the effect of factors on responses. From Figure 3a & b, it can be seen that lag time was increased with increasing the concentration of PG in the formulations while reverse was true for PEG. The significant decrease in lag time (40.9 ± 2.45 min for L_3) was observed when high levels of PG and PEG were used. Figure 4a & b revealed that increasing level of PG in the formulation has positive effect on the K_P while PEG levels did not show any considerable effect, in fact a negative influence can be observed based on regression analysis. However, a gradual increase in the permeation rate of nimesulide was observed with increasing levels of PG and PEG combination in the formulations.

Validation of RSM

In order to validate the model described here, an optimized formulation was predicted from RSM data using numerical optimization provision of software. This was achieved by selecting criteria of attaining minimum value of t_{lag} and maximum value of K_P and ER by applying constraints on Y_1 ($25 \leq Y_1 \leq 60$) and Y_2 ($0.0006 \leq Y_1 \leq 0.001$). This resulted in a

formulation having maximum level of PG (35% w/v) and PEG (35% w/v). Nimesulide lotion formulation was fabricated using the predicted values of enhancers and this was subjected to *in vitro* permeation through rabbit skin using Franz diffusion cells. The resultant t_{lag} and K_P values were 34.6 ± 1.74 (min) and 10.01×10^{-4} (cm/min), respectively. Predicted responses of optimized formulation were 31.2 (min) and 10.24×10^{-4} (cm/min) for t_{lag}, K_P, respectively. The percentage predicted error was less than 10% indicating that the experimental and predicted values were in good agreement ($p < 0.05$) with each other, thus validating the usefulness and predictive ability of RSM.

Conclusion

The present study highlighted the prognostic ability of RSM in optimizing lotion formulations of nimesulide and proved to be a useful statistical tool to study the impact of variables on responses. The findings of this study suggests that the lotion formulation variables have significantly influenced the permeation rate of nimesulide and demonstrated that combination of PG and PEG at equal level resulted in higher permeability of nimesulide. It is difficult to conclude that if these findings are true with other drugs but it is envisaged that the enhancer combination used in this study could produce similar results for other model drugs. Future work would be analyzing the optimized formulation in *in vivo* conditions.

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Conflict of interest

The authors report no declarations of interest.

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Table 1

Lotion Formulation (L)	X_1 : PG	X_2 : PEG	Nimesulide % w/v	Carbopol-940 % w/v	Isopropyl alcohol % w/v	Tween-20 % w/v
L_1	0	0	1	0.2	20	4
L_2	0	-2	1	0.2	20	4
L_3	1	1	1	0.2	20	4
L_4	-1	1	1	0.2	20	4
L_5	0	0	1	0.2	20	4
L_6	0	0	1	0.2	20	4
L_7	1	-1	1	0.2	20	4
L_8	0	0	1	0.2	20	4
L_9	0	2	1	0.2	20	4
L_{10}	-1	-1	1	0.2	20	4
L_{11}	2	0	1	0.2	20	4
L_{12}	0	0	1	0.2	20	4
L_{13}	-2	0	1	0.2	20	4

Factors	Levels used, actual (coded)				
	Very low (-2)	Low (-1)	Medium (0)	High (1)	Very high (2)
X_1 = PG %w/v	7	14	21	28	35
X_2 = PEG %w/v	7	14	21	28	35

Response Variables

Y_1: Lag time (t_{lag})

Y_2: Permeability coefficient (K_p)

Table 2

Solvents	Solubility (mg/mL)	Enhancement factor
Water	0.009 ± 0.001	-
PBS	0.033 ± 0.004	3.7
Methanol	4.950 ± 0.600	550
PBS + methanol (1:1 v/v)	1.100 ± 0.100	122
PG	1.715 ± 0.090	190.5
PEG 400	61.78 ± 3.140	6864

Table 3

Formulations	Viscosity (dynes.s/cm^2)	Spreadability (mg.cm/s)	t_{lag} (min)	J (µg/cm^2/min)	K_P (cm/min) $\times 10^{-4}$	*ER*
L_1	98×10^{-2}	3.71 ± 0.41	93.7 ± 13.6	153.9 ± 5.96	7.7 ± 0.3	2.89
L_2	91×10^{-2}	4.72 ± 0.11	62.3 ± 16.7	133.5 ± 2.74	6.7 ± 0.2	2.51
L_3	101×10^{-2}	3.35 ± 0.39	40.9 ± 2.45	180.5 ± 15.9	9.1 ± 0.8	3.39
L_4	99×10^{-2}	3.48 ± 0.14	109.9 ± 11.8	118.6 ± 2.80	6.0 ± 0.1	2.22
L_5	98×10^{-2}	3.73 ± 0.92	93.4 ± 13.1	158.4 ± 5.16	7.7 ± 0.3	2.97
L_6	98×10^{-2}	3.63 ± 0.67	94.0 ± 12.5	154.2 ± 5.08	7.7 ± 0.3	2.89
L_7	92×10^{-2}	4.69 ± 0.78	67.8 ± 9.69	149.5 ± 1.14	7.5 ± 0.1	2.81
L_8	98×10^{-2}	3.75 ± 0.52	99.9 ± 18.6	151.7 ± 5.66	7.6 ± 0.3	2.85
L_9	109×10^{-2}	3.01 ± 0.21	65.0 ± 18.5	145.7 ± 2.20	7.3 ± 0.1	2.73
L_{10}	93×10^{-2}	4.65 ± 0.13	85.2 ± 15.9	143.3 ± 1.30	7.2 ± 0.1	2.69
L_{11}	96×10^{-2}	3.84 ± 0.09	97.6 ± 9.19	144.1 ± 3.27	7.3 ± 0.2	2.70
L_{12}	98×10^{-2}	3.76 ± 0.32	98.2 ± 8.44	150.2 ± 5.83	7.5 ± 0.4	2.82
L_{13}	95×10^{-2}	3.91 ± 0.49	88.5 ± 7.45	133.7 ± 11.8	6.7 ± 0.6	2.51
L_C	48×10^{-2}	4.87 ± 0.92	110.2 ± 11.1	53.3 ± 0.73	2.7 ± 0.1	-

Table 4

Regression Coefficient	Coefficient Estimate	
	t_{lag}	K_P
β_0	92.7	7.68
$\beta_1(X_1)$ PG	-5.68	0.38
$\beta_2(X_2)$ PEG	0.27	0.13
$\beta_{12}(X_1X_2)$	-12.9	0.70
$\beta_1^2(X_1^2)$	-0.88	-0.15
$\beta_2^2(X_2^2)$	-8.2	-0.16
Model (*p* value)	0.000	0.031
R^2	0.583	0.776
Adjusted R^2	0.485	0.616
PRESS	17915	13.74
F-value	69.23	56.66
Lack of fit (*p* value)	0.09	0.913

Figure 1

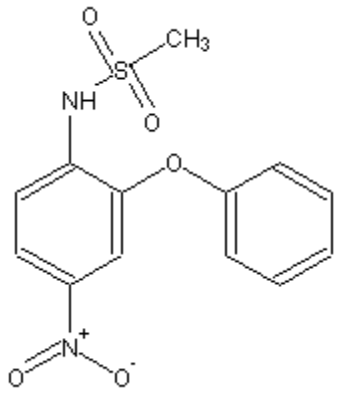
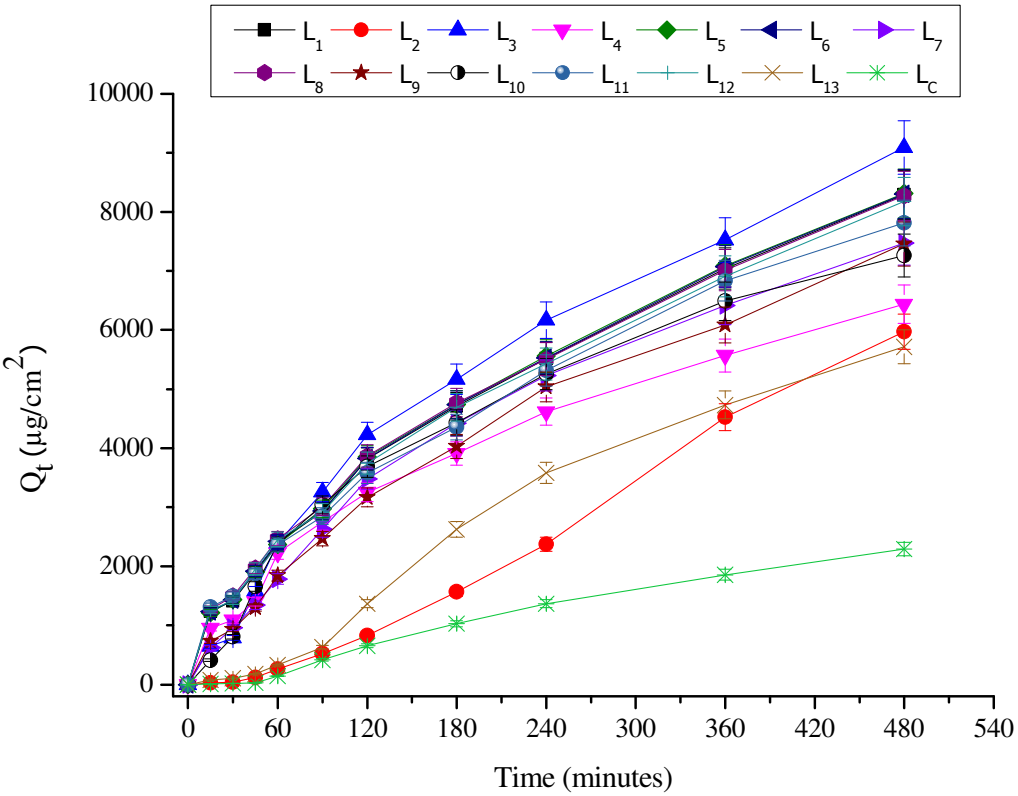


Figure 2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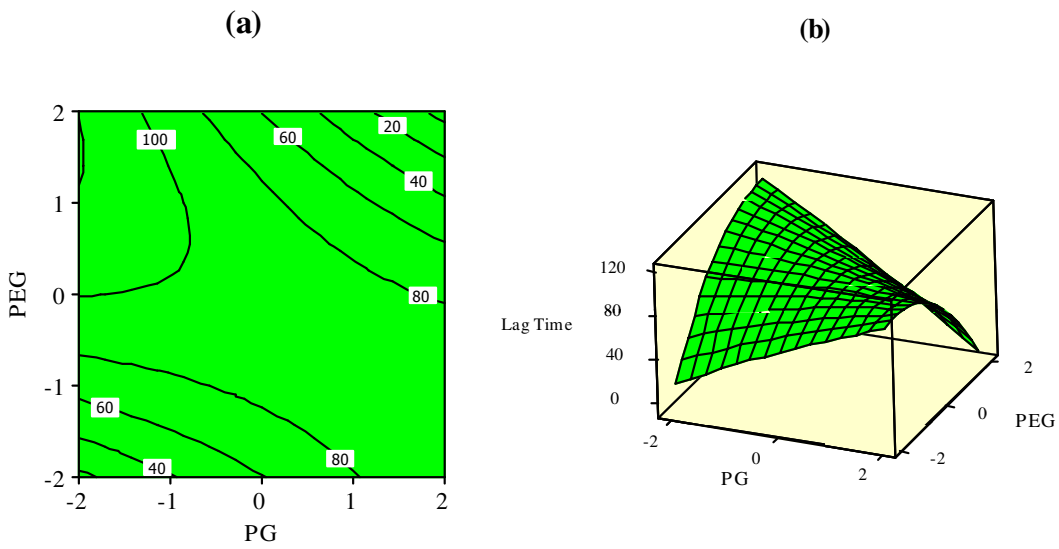
L2
L3
L4
L5
L6
L7
L8
L9
L10
L11
L12
L13
LC
10000
8000
6000
4000
2000
0
Qt (µg/cm2)
0
60
120
180
240
300
360
420
480
540
Time (minutes)

Figure 3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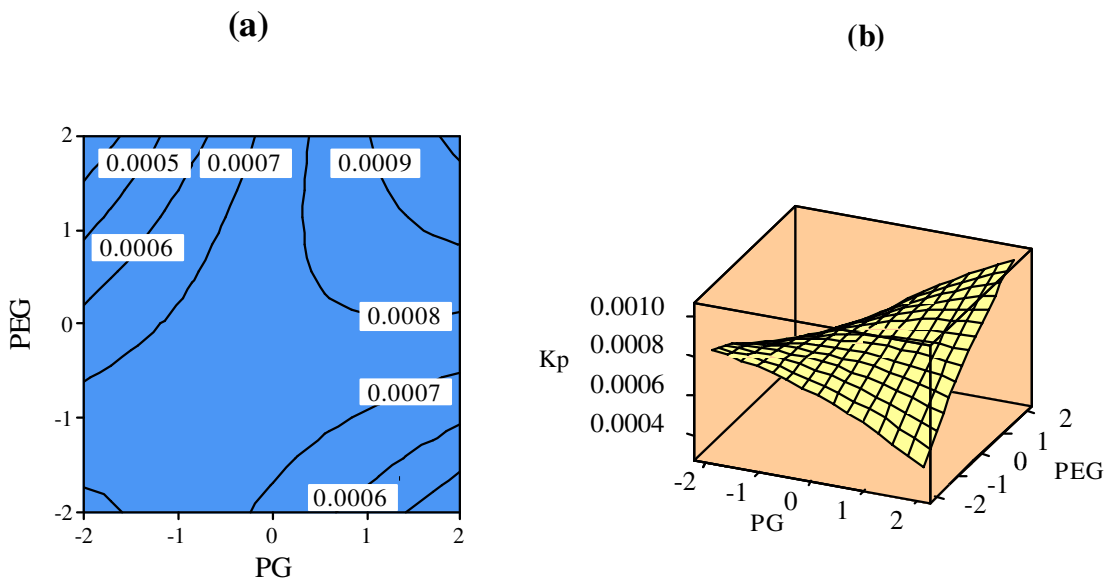
(b)
PEG
PG
100
60
20
40
80
60
40
80
2
1
0
-1
-2
-2
-1
0
1
2
Lag Time
120
80
40
0
-2
0
2
PG
2
0
-2
PEG

Figure 4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(b)
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0.0007
0.0009
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0.0008
0.0007
0.0006
PEG
PG
2
1
0
-1
-2
Kp
0.0010
0.0008
0.0006
0.0004
PG
PEG

Tables Legend

Table 1: Factors in Central Composite Design (CCD) for nimesulide formulations

Table 2: Solubility of nimesulide in different solvents (mean ± S.D.; $n = 3$)

Table 3: Viscosity, spreadability and permeation profile of the nimesulide containing lotions (mean ± S.D.; $n = 5$)

Table 4: Summarized statistical parameters of each response variable determined by multiple regression analysis

Figures Legend

Figure 1: Structure of nimesulide (Courtesy of ACD I-Lab 2.0)

Figure 2: Cumulative amount of drug permeated from nimesulide containing lotion

Figure 3: Estimated contour plot (a) and response surface (b), illustrating the relationship between the permeation enhancers and the lag time.

Figure 4: Estimated contour plot (a) and response surface (b), illustrating the relationship between the permeation enhancers and the permeability coefficient.