ELSEVIER

International Journal of Pharmaceutics 169 (1998) 143–154

international journal of pharmaceutics

Optimization of in vitro nifedipine penetration enhancement through hairless mouse skin

Emilio Squillante *, Anita Maniar, Thomas Needham, Hossein Zia

Department of Applied Pharmaceutics, University of Rhode Island, Kingston, RI 02881, USA

Received 16 September 1997; received in revised form 26 February 1998; accepted 27 February 1998

Abstract

The ability of penetration enhancer/solvent combinations of homologous members of alcohols and alkanoic acids in combination with Azone and isopropyl myristate to accelerate delivery rates of nifedipine was evaluated. Several alkanols and alkanoic acids were found to enhance the in vitro permeation of nifedipine (N) through excised hairless mouse skin. A McLean Anderson experimental design permitted the results of the permeation studies to be interpreted in a stoichiometric fashion. The permeation process was modeled so that simultaneous predictions of the flux or lag time could be made. Regression analysis identified positive synergistic interactions among the formulation components propylene glycol (PG), *cis*-oleic acid (OA), and dimethyl isosorbide (DI)—which strongly affected nifedipine (N) permeation. Of the mixtures tested, a mixture of PG, OA, and DI yielded optimal flux and lag time. Flux values fourfold higher than that required to deliver an equivalent oral daily dose in man were observed with hairless mouse skin. These results suggest that it may be feasible to percutaneously deliver clinically useful amounts of N by the judicious choice of vehicle composition.

Keywords: Penetration enhancer; Nifedipine; Optimization; Mixture Experimental Design

1. Introduction

Selection of components is the single most important decision in the design of topical drug delivery systems. Although information of this nature is crucial in the early phases of dosage form design it remains unclear how to determine a priori the optimal features required in a transdermal delivery system to best overcome the barrier properties of skin. An initial oral adult dose of nifedipine (N) is 30 mg per day. Assuming that approximately 60% of an oral dose is bioavailable, for a transdermal device of 20 cm^2, one would expect that a viable transdermal dosage form must deliver N at rates in excess of 37.5 $\mu g\ h^{-1}\ cm^{-2}$. Even under optimal thermodynamic conditions favoring its diffusion from donor to

* Corresponding author. Tel: + 1 401 8742152; fax: + 1 401 8742152.

0378-5173/98/$19.00
PII S0378-5173(98)00110-0

receptor in vitro, N does not penetrate the skin at rates sufficient to permit clinically useful transdermal application, presumably due to its poor solubility in both lipophilic and hydrophilic media (Squillante et al., 1997). Results of these solubility determinations show that N is poorly soluble in both lipophilic and hydrophilic solvents. It was concluded that the solubility profile of N is such that penetration into the deeper layers of the skin is not favorable due to the markedly poor affinity for systems structured by extensive hydrogen bonding.

Conventional wisdom suggests that chemical and physical enhancers possess distinct mechanisms of action which promote drug penetration by altering either the polar, nonpolar, or polar/nonpolar pathways (Shah, 1993). To illustrate: the penetration enhancement effects of OA, which act selectively on the extracellular lipids by lipid fluidization and lipid phase separation (Naik et al., 1995), are restricted to the outermost layers of skin. Thus, optimal results with penetration enhancers such as OA on very lipophilic drugs may require additional formulation ingredients with complementary action that both promote not only penetration *into* but also permeation *through* the skin. Thus, while a certain enhancer might be considered appropriate for polar compounds, that enhancer might be considered less appropriate for nonpolar permeants. A conundrum arises in the case of N, as it falls into neither class. Our approach was to combine enhancers of disparate actions that could act in a positive, synergistic fashion to enhance N permeation rates.

Visualization of the permeation process as an intimate relationship between a finite ratio of formulation components may offer insight into the mechanism of the transdermal diffusion process. Unfortunately, it is difficult to visualize spatially the effect of a change in multiple variables (i.e., formulation ingredients) on the measured response. This renders the interpretation of the results of experiments involving multi-component mixtures difficult. Response surface methodology provides a tool by which formulation components may be optimized with respect to N permeability and lag time. A distinguishing feature of these designs is that a change in proportion of one component requires collateral change in the next. Rather than unrestrained amounts, the mole fraction ratios of donor ingredients are non-negative, independent, controllable factors which must sum to unity. These seemingly intractable qualities may be resolved using Mixture Experimental Designs (Cornell, 1983). We employ various facilities available in the SAS system in conjunction with modified McLean and Anderson experimental designs to optimize levels of formulation components which maximize flux yet minimize lag time.

2. Materials and methods

2.1. Materials

Azone (Whitby Research, Richmond, VA), butanol, decanol, dimethyl isosorbide (ICI, Wilmington, DE), dimethylsulfoxide, dodecanol, elaidic acid, ethanol, isopropyl myristate, isopropyl palmitate, linoleic acid, linolenic acid, methanol, myristic acid, 1-nonanol, *cis*-oleic acid, oleyl alcohol, palmitic acid, palmitoleic acid, polyethylene glycols (Dow Corning, Midland, MI), propylene glycol, stearic acid, and undecylenic acid were obtained from Sigma (St. Louis, MO) unless specified otherwise. All other chemicals were reagent grade.

2.2. Methods

2.2.1. Preparation of suspensions of N

Formulations were prepared and measured on a molar basis. The first series of experiments consisted of N suspended in the pure solvent vehicles listed in Table 1. Another series of experiments were designed using N in cosolvent suspensions consisting of dimethyl isosorbide (DI), propylene glycol (PG), or polyethylene glycol 400 (PEG400) as the base solvent. For example, binary donor suspensions were prepared by combining the long chain acids and alcohols in DI as the base solvent (enhancer/base solvent ratio was 1:9). The compositions of these binary systems are listed in Table 2. More complicated mixtures of DI and PG were prepared by inclusion of isopropyl myristate (IP), ethanol (ET), *cis*-oleic acid (OA), and Azone

Table 1
Solubility parameter (δ), saturation solubility of nifedipine (C_s), nifedipine flux (J) and permeability coefficient (P) of nifedipine suspended in pure base solvents

Vehicle	δ (cal cm^{-3})$^{1/2}$	C_s (mg ml^{-1})	J (μg cm^{-2} h^{-1})	P[a] (cm h^{-1}, $\times 10^5$)
Dimethyl isosorbide	9.0	84	2.14	2.6
Isopropyl myristate	8.0	2.0	0.61	30.5
cis-Oleic acid	7.9	0.33	3.29	997.0
Polyethylene glycol	11.7	80.5	0.05	0.1
Propylene glycol	14.8	14.0	2.83	20.2
Methanol	14.5	31.2	2.2	7.1
Ethanol	12.8	19.1	2.7	14.1
Butyl	11.2	15.5	0.3	1.9
Isobutyl	10.3	16.0	0.5	3.1
Octanol	10.3	8.1	0.6	7.4
Nonanol	10.5	7.7	0.8	10.4
Decanol	9.8	6.7	0.3	4.5
Dodecanol	9.1	6.0	4.2	7.0
Oleyl	8.9	7.1	2.0	27.8

[a] Calculated from Eq. (1).

(AZ). These more complicated mixtures, listed in Table 3, show the composition of the 15 possible quaternary mixtures of PG, IP, OA, ET, AZ and DI prepared such that the mole fraction of each component in a given formulation was 25 mol%. In all cases, a moderate (10%) excess of N was added to the cosolvent mixtures and the suspension thus obtained were agitated for 72 h at 37°C. The saturation solubility of N in each system was determined after filtration through 0.22-μm filters. The remaining unfiltered suspensions were employed as donor formulations in the permeation experiments.

2.2.2. Diffusion cell experiments

Permeation data were obtained in vitro using vertical diffusion cells with an area of permeation of 4.9 cm^2 (i.d. = 25 mm) (Vanguard, Neptune, NJ). The membrane employed was fresh abdominal female hairless mouse skin (26–28 g, 8–12 weeks of age) bred from the progeny of four females and one male SKH-1 obtained from Charles River Laboratories. Sink conditions were maintained by periodically introducing 17 ml of fresh receptor solution composed of 40% (v/v) PEG400 in 0.9% sodium chloride at sample times. Samples of 0.3 ml were withdrawn at intervals over 24 h. Cosolvent donor formulations (10 μmol) were applied to the epidermal side of the skin in the donor compartment using tared syringes. At the selected time intervals the receptor solution was assayed for drug content and the amount of drug withdrawn was corrected in the calculation of the cumulative amount released. Identification of N breakdown products and quantitation of N concentrations were measured by an HPLC method (Squillante et al., 1997).

Penetration curves were constructed by plotting the cumulative amount of N measured in the receptor phase (Q) against time (Q vs. t). Only the linear portion of the plot with a correlation coefficient greater than 0.90 throughout the linear phase was considered indicative of steady state penetration and used for lag time and flux determinations. Lag times were estimated by back extrapolation of the linear portion to the abscissa. When the permeation curve was well defined, linear regression analysis yielded slopes that, when divided by the area (4.9 cm^2) yielded the steady state flux value in μg h^{-1} cm^{-2}. The permeation study results are the average of three replicates unless otherwise noted. Permeability coefficients were determined by dividing the slope obtained from the linear portion of the Q versus t profile by the corresponding solubility of N in that solvent

Table 2
Effect of alkyl chain length on skin permeation of nifedipine from binary mixtures containing base solvent plus alkanoic acids at the 10 mol% level

Alkanoic acid	δ (cal cm^{-3})$^{1/2}$	C_s (mg ml^{-1})	J (μg cm^{-2} h^{-1})	E.R.	T_l
Polyethylene glycol 400-based mixtures					
Myristic	10.4	86	0	0	24
Palmitic	10.5	139	0	0	24
Undecylenic	10.9	63	0	0	24
Linoleic	9.8	102	11	220	10
Linolenic	10.2	97	3	60	14
cis-Oleic	10.3	106	20	1200	10
Propylene glycol-based mixtures					
cis-Oleic	13.4	8	34	12	5
Undecylenic	13.6	13	11	4	4
Myristic	10.8	39.6	0	0	24
Dimethyl isosorbide-based mixtures					
Palmitic	9.8	32.3	0	0	24
Linoleic	8.8	38.7	12	6	1
cis-Oleic	9.7	34.5	15	7	2
Undecylenic	9.1	24.0	0	0	24

$$P = \frac{\frac{dQ}{dt}}{(AC_s)} \quad (1)$$

where P = permeability coefficient (cm h^{-1}), dQ/dt = the slope of the straight portion of the penetration curve (μg h^{-1}), A = the surface area (4.9 cm^2) and C_s = the equilibrium saturation concentration of drug in the donor phase (mg cm^{-3}). The Enhancement Ratio relative to pure solvent ($ER_{solvent}$) was used to compare N flux among formulations (for example, ER_{DI} designates the ratio of flux of N from cosolvent mixture relative to flux of N from pure DI). Mean flux and lag time were compared by ANOVA using Tukey's method at the 95% level.

Mass balance was performed by washing the donor compartments with 10 ml of methanol each, taking care to remove any solid N particles and to keep the time of contact of methanol to a minimum (less than 3 min total). The methanol washes were combined, diluted and measured by HPLC.

2.2.3. Experimental design

Initial experiments (described below) showed that mixtures of propylene glycol (X_1), *cis*-oleic acid (X_2), ethanol (X_3) and dimethyl isosorbide (X_4) had a positive effect on N flux and lag time and various mole fraction combinations of these materials were examined in detail. The experimental design, designated 'Design I', conforms to a two-step procedure described in the literature (McLean and Anderson, 1966; Cornell, 1983). Mixtures were prepared in a manner such that their proportions are non-negative, sum to unity, and are restricted to the following upper and lower bounds:

$10\% < X_1 < 72\%$
$8\% < X_2 < 25\%$
$10\% < X_3 < 25\%$
$10\% < X_4 < 70\%$

The design points comprise the vertices and centroids of a convex polyhedron suitable for fitting first-degree and quadratic models in the constrained mixture space. These constraints were subsequently tightened in a second series of experiments, designated as 'Design II.' Experimental design, analysis and optimization were performed using the ADX™ menu system of SAS/QC and SAS/STAT software, Version 6.0 (Statistical Quality Improvement R&D Applications Division, SAS, Carey, NC). Regression analyses of data obtained from permeation experiments were employed to identify positive synergistic interactions among formulation components.

Table 3
Nifedipine flux (J) from various quaternary donor cosolvent systems prepared by blending propylene glycol (PG), isopropyl myristate (IP), *cis*-oleic acid (OA), Azone (AZ), ethanol (ET), or dimethyl isosorbide (DI), each at the 25 mol% level

Code	Composition (mol%)						J (μg h^{-1} cm^{-2})
	PG	IP	OA	AZ	ET	DI	
A1	25	25	25	25			2.3 ± 3
A2		25	25	25	25		28.1 ± 4
A3			25	25	25	25	6.1 ± 3
A4	25		25	25	25		4.5 ± 1
A5		25		25	25	25	70.8 ± 4
A6	25		25		25	25	120.0 ± 10
A7	25	25		25		25	15.0 ± 3
A8	25	25	25		25		15.1 ± 2
A9		25	25	25		25	2.5 ± 0.4
A10	25			25	25	25	28.1 ± 3
A11	25	25			25	25	NA[a]
A12	25	25	25			25	41.8 ± 3
A13	25	25		25	25		69.5 ± 14
A14		25	25		25	25	5.9 ± 4
A15	25		25	25		25	14.9 ± 3

[a] Formulation A11 resulted in two immiscible phases.

2.2.4. Solubility parameters

The solubility parameters were obtained using the method illustrated by Sloan et al. (1986) and were used to predict the solubility of N in the various mixtures.

3. Results and discussion

3.1. Permeation studies

N fluxes calculated from the steady state portion of the Q vs. t profiles obtained following application of N suspended in pure ET, IP, OA, PG, or DI are shown in Table 1. N flux was generally poor when applied singly which showed that no individual solvent possessed the necessary intrinsic activity to dramatically promote N permeation. However, when used in conjunction with each other, instances of synergism were noted. A selection of the physical and laboratory data from the solubility and permeation studies performed on alcohols is shown in Table 1. Similar data for donor suspensions prepared from the binary acid/DI-cosolvent systems are presented in Table 2. The relative effects of the quaternary cosolvent mixtures on N flux are shown in Table 3.

The histogram in Fig. 1 compares enhancement potential of equimolar (10 μmol) amounts of long-chain acids in DI. In general, an increase in chain length enhanced N permeation in the series of acids and alcohols studied, with maximum N permeation occurring from DI-based suspensions with 10% *cis*-oleic acid ($ER_{DI} = 7$). Also shown are the effects of Azone on these systems. Unsaturated, long-chain acids had pronounced effects which were restricted to the *cis*-unsaturated fatty acids, as they were not observed for their saturated or *trans*-unsaturated (elaidic) acid counterparts. On a molar basis, *cis*-oleic acid was found to be the most potent enhancer.

These results prompted a more thorough study of the enhancing properties of *cis*-oleic acid to determine the best choice of cosolvent vehicle(s) to maximize the N. Aungst et al. (1986) had shown that the effects of a variety of penetration enhancers on naloxone absorption are vehicle-dependent. Wotton et al. (1985) demonstrated that the choice of vehicle was an important factor and that PG was necessary for promoting the effects of Azone on the permeation of metronidazole; similar effects have been reported for trifluorothymidine (Sheth et al., 1986). An unresolved

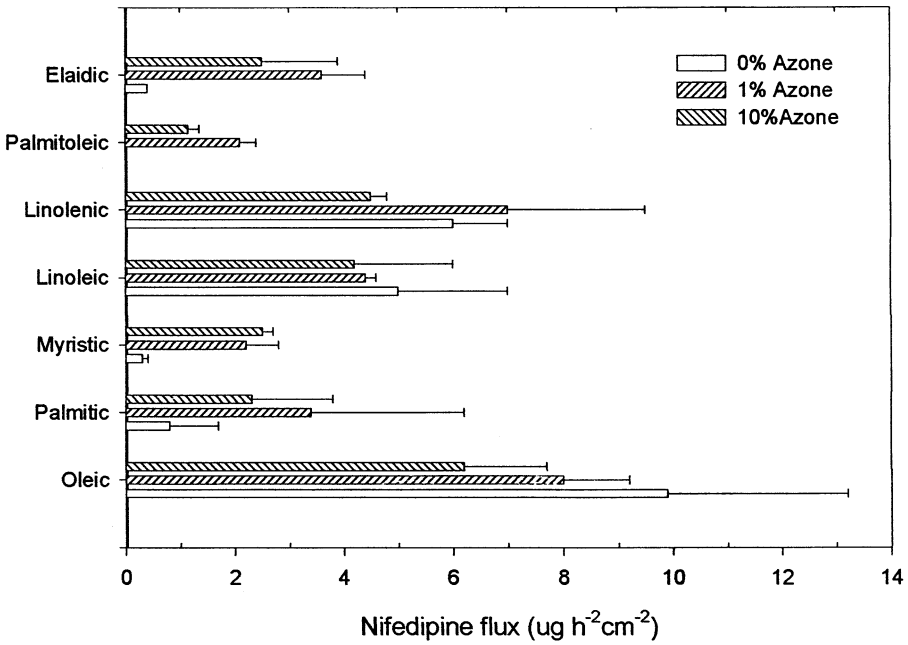

Fig. 1. Influence of Azone and various acids (10 mol% acid) in dimethyl isosorbide on nifedipine flux through hairless mouse skin.

question on this point was the function of the vehicle in the overall drug permeation mechanism, namely does the vehicle enhance N flux viz. effect on drug saturation solubility (i.e., drug thermodynamic activity and chemical potential) or does the vehicle exert an intrinsic effect on the barrier properties of the skin. In this study PEG400, PG and DI were chosen as formulation components to help distinguish the nature of effect between enhancer and cosolvent vehicle. All three liquids had been determined to be good solvents for N (Table 1). However, previous studies had shown that topical application of suspensions of N in pure PEG400 had little effect on N permeation. In fact, when N suspended in PEG400 was reapplied to the skin after a 24-h washout period, N flux remained very low and on this basis PEG400 was considered to be a 'noninteracting' vehicle. Suspensions of N in DI did show some enhancement of N permeation and DI was classified as an 'interacting' vehicle (Squillante, 1993).

The present in vitro permeation experiments show that binary mixtures of both DI and PEG400 in the presence of *cis*-oleic acid had a positive effect on penetration of N. Fig. 2 shows the amount of N permeated versus time from formulations containing various levels of OA in PEG400. These results were less satisfactory com-

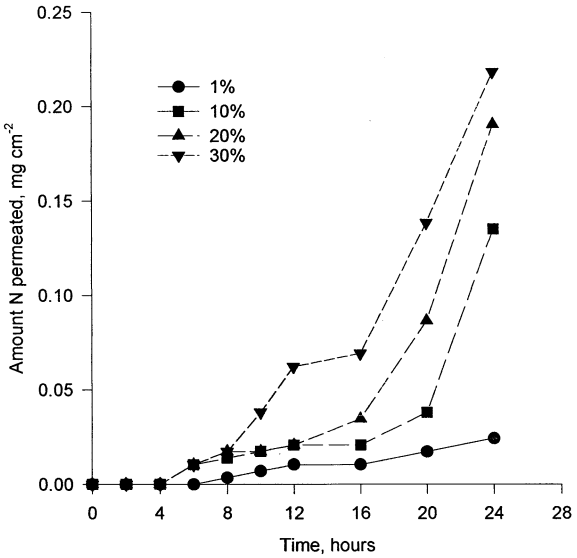

Fig. 2. Effect of various concentrations of *cis*-oleic acid in polyethylene glycol 400-based donor formulations on nifedipine permeation.

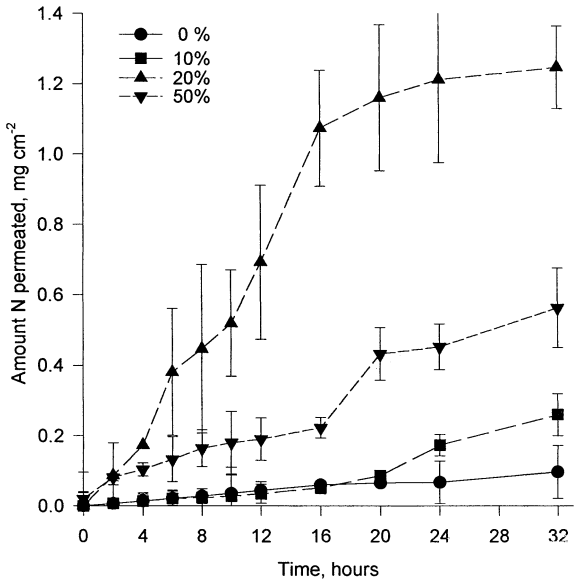

Fig. 3. Effect of various concentrations of *cis*-oleic acid in dimethyl isosorbide-based donor formulations on nifedipine permeation.

pared to those obtained following application of 1%, 10%, 20%, 30%, or 50% OA in DI-based cosolvent systems. A selection of these data, obtained following application of 10%, 20%, and 50% OA in DI, with pure DI included for comparison, is shown in Fig. 3. For the DI-based suspensions containing 0–50% OA, optimal flux values were obtained with the formulation containing 20% (w/w) *cis*-oleic acid ($ER_{DI} = 20$). N permeation from the DI-based suspensions in Fig. 3 are sixfold greater than from the PEG400-based suspensions of Fig. 2. Comparison of Figs. 2 and 3 shows that the intensity of enhancement was dependent upon choice of the solvent vehicle. A second finding that emerged was that N solubility was of no major consequence in the overall performance of the different mixtures. When *cis*-oleic acid was added to either an interacting (e.g., DI) or a non-interacting (e.g., PEG400) base-solvent, the resulting binary systems thus formed had higher saturation solubilities for N (Table 2) but very different effects on N permeation. On the other hand, the binary systems of OA in PG in Table 2 had comparatively low N saturation solubility (8 mg ml^{-1}) yet promoted flux to a much greater degree with shorter lag times than corresponding OA in DI systems, which had comparatively higher N saturation solubility (34.5 mg ml^{-1}). Although the N fluxes listed in Tables 1 and 2 are generally poor, it was noted that these results were obtained with saturated acids and alcohols with molecular weights proximal to myristic acid. The second and third highest flux was derived from formulations containing the isopropyl ester of myristic acid, suggesting that this structure might be important to N flux. Also, IP, like PG, did not solubilize N as well as DI and PEG400 or even PG, yet N flux enhancement was observed from these systems. Thus, it was concluded that factors in addition to drug solubility were responsible for the drug permeation enhancement properties of OA in combination with poorer solvents.

The data in Table 3 summarize the intrinsic activity on N permeation enhancement from the 15 possible quaternary blends. The effects of AZ, ET, and IP on N flux was less clear cut than the data obtained from the binary acid experiments in Table 2. The mixtures containing AZ and IP had variable effects on the N flux shown in Table 3. Mixtures of PG and DI with AZ or IP offered little improvement in N permeation rates compared to mixtures of OA or ET in PG or DM. Although AZ and IP enhanced N flux, a decision was made to focus attention on mixtures containing ET, OA, DM and PG. Formulations without DI showed low N flux compared with those with DI present ($p < 0.05$). Mixtures pairing PG with DI (e.g., A6) displayed ER_{DI} five times greater than those not containing these components. While AZ appears to have little enhancing effect, as seen in formulations A1 (PG/IP/OA/AZ), other formulations combining AZ with DI e.g., A7 (PG/IP/AZ/DI) and A10 (PG/AZ/ET/DI) showed some improvement of N flux, suggesting to us that DI may exert its effect by enhancing the activity of other enhancers. Mixtures containing PG, ET or DI displayed the highest overall enhancement factors. The synergistic relationship among these three components was highest in the presence of OA (A6). A maximum flux of 120.2 ± 10.3 $\mu g\ h^{-1}\ cm^{-2}$ was observed from formulation A6 consisting of PG/OA/ET/DI, each at the

Table 4
Design I: Nifedipine lag time (T_l) and flux (J) from quaternary systems for a four-component simplex-centroid design: propylene glycol (PG), *cis*-oleic acid (OA), ethanol (ET), or dimethyl isosorbide (DI) for donor suspensions using the following constraints: PG = 10–75 mol%, OA = 10–36 mol%, ET = 10–36 mol%, DI = 10–75 mol%

No	Composition (mol%)				Response variables	
	PG	OA	ET	DI	T_l (h)	J (μg cm^{-2} h^{-1})
B1	72	10	8	10	2.4 ± 1.0	148.9 ± 60.6
B2	55	25	10	10	2.0 ± 2.2	60.0 ± 25.3
B3	55	10	25	10	1.9 ± 5.7	96.0 ± 53.1
B4	10	10	25	55	3.3 ± 0.8	12.2 ± 2.4
B5	10	10	10	70	4.8 ± 0.3	11.0 ± 0.8
B6	25	10	10	55	3.7 ± 0.4	58.8 ± 45.7
B7	14	36	36	14	3.8 ± 0.6	48.2 ± 7.5
B8	10	25	25	40	7.6 ± 1.0	48.2 ± 5.5
B9	25	25	25	25	2.5 ± 1.4	120.2 ± 10.3

25% level. Synergistic effects observed in mixtures of these solvents, particularly the 25:25:25:25 PG/OA/ET/DI quaternary mixture, show that the effect of one was dependent upon the other to achieve maximal flux. Based on these observations, a decision was made to restrict the focus of the study and optimize only mixtures of PG, OA, ET and DI.

3.2. Optimization

For those N flux values shown in Table 1 obtained when PG, ET, OA, or DI were applied singly, the N in ET suspension displayed the highest enhancement whereas the N in PG suspension showed the lowest enhancement of N permeation. Yet when these solvents were blended, the strong effect of ET was found to be unexpectedly less than predicted from the pure solvent studies. The apparent synergism between OA and the other components prompted another series of experiments in which the ratio of OA to ET was held constant and the proportion of PG to DI was allowed to vary. A portion of the results from these experiments is shown in Table 4. A clear difference in the general shape of the Q vs. t profiles was observed with formulations B1 through B9. As the ratio of PG to DI varied, a monotonic decrease in lag time was observed as the level of DI fell to an optimum of 6%. Both lag time and flux were improved by limiting the level of DI to less than 20%. Permeation rates of N fell rapidly in those formulations in which the level of DI approached 70%. Formulation B5, the formulation containing the highest level of DI, demonstrated the lowest mean N flux.

At this point, it became clear that a complicated, multi-component mixture was necessary to achieve optimal flux and lag times. Furthermore, interpretation of results obtained following manipulation of a multi-component donor would become intractable since the nature and extent of synergism amongst four component PG/ET/OA/DI mixtures requires $n + 1 = 5$ dimension space. As a solution, the apparent synergism(s) among various component(s) were investigated in a series of permeation experiments based on a McLean Anderson mixture design, the results of which are listed in Table 5. These results were interpreted by regression analysis to form a model to describe the simultaneous maximization of flux and minimization of lag time. The parameter estimates for this expression are shown in Table 6. Finally, an empirical test of the predictiveness of the method and corroboration of the predictions made by the parameter estimates in Table 6 were performed in a final set of permeation experiments in which N permeation was evaluated following application of ternary PG/OA/DI mixtures. The composition of these formulation and the permeation results are listed in Table 7.

Table 5
Design II. Nifedipine lag time (T_l) and flux (J) from the four-component simplex-centroid blending propylene glycol (PG), *cis*-oleic acid (OA), ethanol (ET), and dimethyl isosorbide (DI) for donor suspensions keeping [OA] and [ET] $\leq 10\%$

No	Design points[a] (mol%)				T_l (h)	J[b] (μg h^{-1} cm^{-2})
	PG	OA	ET	DI		
C1	97	1	1	1	3.8 ± 0.6	110 ± 16
C2	88	1	1	10	7.6 ± 1.0	106 ± 25
C3	88	10	1	1	2.0 ± 0.5	60 ± 30
C4	79	10	1	10	2.3 ± 0.3	117 ± 10
C5	88	1	10	1	5.1 ± 0.7	80 ± 19
C6	79	1	10	10	4.8 ± 0.4	103 ± 10
C7	79	10	10	1	0.7 ± 0.4	101 ± 2
C8	70	10	10	10	2.4 ± 1.0	149 ± 6
C9	82	6	6	6	3.3 ± 1.3	104 ± 46
C10	88	6	6	1	3	131
C11	79	6	6	10	2	111
C12	88	6	1	6	3	109
C13	79	6	10	6	2	125
C14	88	1	6	6	3	99
C15	79	10	6	6	2	127

[a] Design point nos. C1–C8 represent vertices; no. C9 represents the overall centroid; point nos. C10–C15 represent face centroids.
[b] Mean $\pm$ S.D. of four cells except points C10–C15 which were single determinations as per experimental design.

It is important to note that the expression of formulation constituents as mole fraction percent permits a unique opportunity to model the diffusion process from a stoichiometric perspective. Adoption of the mole fraction reference system permitted the experimental data (in the form of multiple outcomes (flux and lag time) from complicated, multi component mixtures) to be studied in a meaningful way that illuminates the relative importance of the individual components and, more important, better demonstrates the potential interaction between components. The intent was less one of generating a mathematical model or predicting an outcome, but rather an exercise to identify synergy between diverse mechanisms of action. Under the best circumstances, results of permeation studies may be interpreted with an eye toward identification of factors which significantly affect permeation, and selection of levels for these factors which maximize flux yet minimize lag time. The criterion for best fit were consideration of the correlation coefficient, residual pattern, and sum of squared residuals among the various models tested. Formulation changes were based on comparison of each coefficient estimate to the value of its standard error in the form of an approximate Student's t-statistic and then comparing each computed value of t to the tabled value $t_{15,0.25}$.

For the situation in which flux and lag time are considered simultaneously, regression techniques resulted in the model predictive of flux and lag time shown in Table 6. The model shows that N permeation was dependent upon OA and DI, while ET and PG have less activity or are present in excess, respectively. Ethanol was found to be not crucial to superior performance and was removed from the formulation. The model thus obtained predicted that it was not so much the main effects, but rather the OA/DI and OA/PG interaction which explains enhanced permeation. Analysis of the fitted surface yielded negative and positive eigenvalues, predictive of a saddle region of 'good' formulations coinciding with OA at the 10% level. The sign of the βs (negative) and their high magnitude infer that individual components deter N flux. Synergism was defined as a large and positive value of β_{ij} significantly greater than zero. Of the six possible two-way interactions, X_2 (*cis*-oleic acid) strongly interacts with PG and DI.

Table 6
Parameter estimates obtained from simultaneous maximization of N flux and minimization of N lag time for the data presented in Table 5 using the SAS ADX™ system experimental design software

Variable[a]	df	Parameter estimate	Standard Error	t[b]	$P>\|T\|$
X_1	1	100.5	27.1	3.715	0.0016
X_2	1	−3369.0	1588.1	−2.121	0.0480
X_3	1	−1773.4	1605.6	−1.105	0.2839
X_4	1	−146.3	111.1	−1.317	0.2044
$X_1 \times X_2$	1	3990.3	1912.4	2.087	0.0514
$X_1 \times X_3$	1	2098.5	1898.5	1.105	0.2836
$X_1 \times X_4$	1	−14.7	167.3	−0.088	0.9308
$X_2 \times X_3$	1	9573.7	4502.8	2.126	0.0476
$X_2 \times X_4$	1	4658.8	2257.0	2.064	0.0537
$X_3 \times X_4$	1	1945.4	2356.0	0.826	0.4198

[a] X_1 = propylene glycol; X_2 = *cis*-oleic acid; X_3 = ethanol; X_4 = dimethyl isosorbide.
[b] t = computed value of Student's t-statistic (tabled value $t_{15,0.25} = 0.691$).

These interaction terms were large relative to their single factor terms and probably represent the driving force of enhanced N flux for these systems.

The individual three-dimensional response surface for flux, based on Design III, is shown in Fig. 4. Fig. 4 shows the clear optimum in the N flux response surface observed for the formulations tested in Design III. N flux was very sensitive to the level of DI and was influenced to a lesser extent by the level of OA. Over the range of constraints shown in Fig. 4 the measured N flux was lowest from mixtures with low DI and OA concentrations. The steep rise in the N flux surface portrays the dramatic enhancing effect of OA and DI on N flux from the PG-based vehicles. As the mole fraction of OA and DI increased, N flux was promoted up to this optimum and then fell off as the mole fraction of OA and DI components exceeded this optimal level.

The individual three-dimensional response surface for the lag time response, based on Design III, is presented in Fig. 5. The shape of the N lag time surface was less sensitive to the level of OA

Table 7
Design III: Skin kinetics of nifedipine through hairless mouse skin for propylene glycol-based ternary cosolvent systems with various *cis*-oleic acid to dimethyl isosorbide (OA/DI) molar ratios

ID	OA/DI	J (mg h^{-1} cm^{-2})	T_l (h)	C_s (mg ml^{-1})	Amount N delivered (mg)
D1	4:2	$0.1037 \pm 2.5e^{-3}$	3.2	7.45 ± 0.01	12.2[a]
D2	10:2	$0.1692 \pm 3.7e^{-3}$	2.6	10.27 ± 0.03	19.9
D3	4:10	$0.2239 \pm 9.3e^{-3}$	5.3	17.80 ± 0.02	26.4
D4	10:10	$0.2010 \pm 9.5e^{-3}$	7.0	13.11 ± 0.01	23.7
D5	4:6	$0.1791 \pm 4.3e^{-3}$	3.3	13.66 ± 0.06	21.1
D6	10:6	$0.1356 \pm 2.69e^{-3}$	3.4	10.07 ± 0.06	16.0
D7	4:6	$0.2118 \pm 5.7e^{-3}$	5.7	12.42 ± 0.02	25.0
D8	10:6	$0.2172 \pm 4.9e^{-3}$	3.6	13.27 ± 0.01	25.6
D9	7:2	$0.1064 \pm 6.0e^{-3}$	1.9	8.13 ± 0.01	12.5
D10	7:10	0.2040[b]	4.9	13.86 ± 0.12	24.0
D11	7:2	$0.1817 \pm 2.8e^{-3}$	2.5	10.83 ± 0.04	21.4
D12	7:10	$0.2045 \pm 7.1e^{-3}$	5.0	13.43 ± 0.03	24.1
D13	7:6	$0.2216 \pm 8.8e^{-3}$	2.8	14.45 ± 0.03	26.1

[a] Corresponds to amount of N that will permeate 20 cm^2 in 24 h.
[b] Single determination.

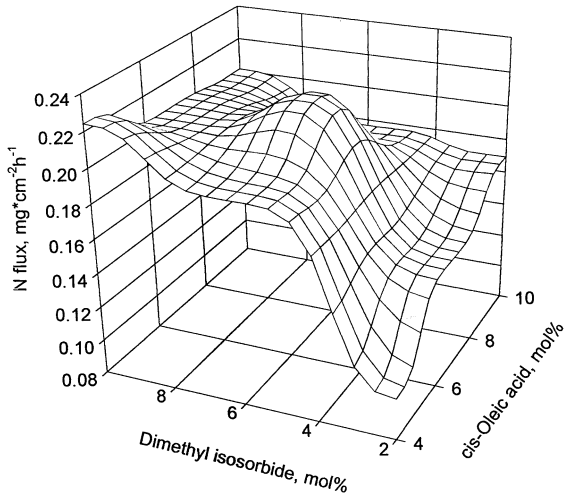

Fig. 4. Response surface showing the nifedipine flux as a function of the mole fraction percent of the *cis*-oleic acid and of dimethyl isosorbide added to propylene glycol-based donor suspensions of nifedipine.

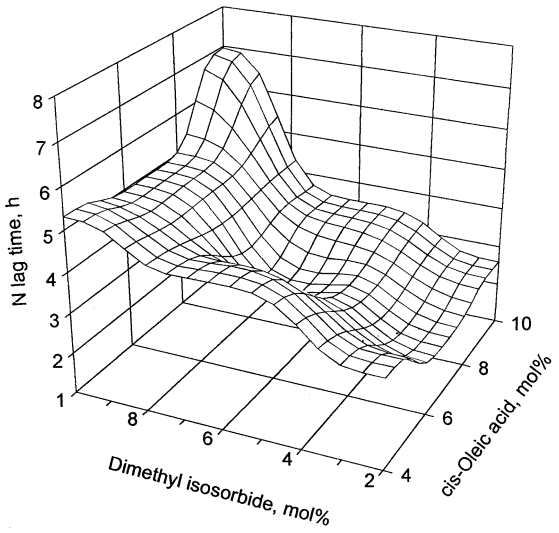

Fig. 5. Response surface showing the lag time as a function of the mole fraction percent of the *cis*-oleic acid and of dimethyl isosorbide added to propylene glycol-based donor suspensions of nifedipine.

and DI than the N flux surface. In this case no clear optimum lag time was observed. The general trend was a direct relationship between N lag time and the mole fraction of the OA and DI components. Lag time values decreased gradually in formulations consisting of $<9\%$ OA and $<7\%$ DI, whereupon the slope of the response surface increased steeply. Formulations containing $>10\%$ OA and $>8\%$ DI had unacceptable lag times. Of the formulations tested in Design III, formulation D13 was considered to be the best compromise between the two response variables of flux and lag time.

4. Conclusions

Unsaturated, long-chain acids had pronounced effects on N permeation rate and lag times. These effects were restricted to the *cis*-unsaturated fatty acids. In particular, the efficiency of binary mixtures of PG and DI as permeation enhancers of N is magnified in the presence of OA. Response surface methodology proved to be a useful tool in the investigation of the influence of individual and synergistic vehicle effects on total formulation performance. Ternary mixtures possessed maximal flux and minimal lag times as compared to those obtained when these materials are applied singly or as binary mixtures. This rather novel approach facilitated the rational selection of vehicle components which, in vitro, demonstrated flux and lag times on the order of those required to develop a transdermal dosage form. Finally, a synergistic relationship exists between N and the suspending vehicle. Further studies to establish the nature of this dependency are presently under way.

Acknowledgements

The authors acknowledge the interest and support of Dr Rainer Hoffman and the partial support by a grant from Lohmann Therapie-Systeme, GmbH & Co.

References

Aungst, B.J., Rogers, N.J., Shefter, E., 1986. Enhancement of naloxone penetration through human skin *in vitro* using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–234.

Cornell, J.A., 1983. Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data. Wiley, New York.

McLean, R.A., Anderson, V.L., 1966. Extreme vertices design of mixture experiments. Technometrics 8, 447–454.

Naik, A., Pechtold, L.A., Potts, R.O., Guy, R.H., 1995. Mechanism of cis-oleic acid-induced skin penetration enhancement in vivo in humans. J. Control. Release 37, 299–306.

Shah, V.P., 1993. Skin penetration enhancers: Scientific perspectives. In: Hsieh, D.S. (Ed.), Drug Permeation Enhancement Theory and Applications. Marcel Dekker, New York.

Sheth, N.V., Freeman, D.J., Higuchi, W.I., Spruance, S.L., 1986. The influence of Azone, propylene glycol and polyethylene glycol on *in vitro* skin penetration of trifluorothymidine. Int. J. Pharm. 28, 201–209.

Sloan, K.B., Koch, S.A.M., Siver, K., Flowers, F.P., 1986. Use of solubility parameters of drug and vehicle to predict flux. J. Invest. Dermatol. 87, 244–252.

Squillante, E., 1993. Physicochemical properties affecting the transdermal permeation of nifedipine. Ph.D. Thesis, University of Rhode Island, USA.

Squillante, E., Needham, T., Zia, H., 1997. Solubility and *in vitro* transdermal permeation of nifedipine. Int. J. Pharm. 159, 171–180.

Wotton, P.K., Mollgaard, B., Hadgraft, J., Hoelgaard, A., 1985. Vehicle effect on topical drug delivery. III. Effect of azone on the cutaneous permeation of metronidazole and propylene glycol. Int. J. Pharm. 24, 19–26.