Formulation and optimization of microemulsion-based organogels containing propranolol hydrochloride using experimental design methods

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ABSTRACT

Background and the purpose of the study: Lecithin organogels are formed spontaneously by adding a given amount of water to lecithin/organic solvent mixture. The aim of this research was to develop and optimize a semisolid preparation with appropriate release profile.

Methods: Lecithin organogels containing Propranolol hydrochloride (PR) were formulated, based on phase diagram studies, using soybean lecithin (Epikuron 200), isopropyl myristate (IPM) and propranolol hydrochloride (PR) solutions (10, 20, 30, 50 % w/w) or water at various lecithin/ IPM weight ratios. The flux and the viscosity of the prepared formulations were determined and further chosen as two responses for optimization, using experimental design and optimization methods (i.e. Modified Simplex and Central Composite Designs, respectively). Results of modified simplex runs (i.e. lecithin: 30-50%, PR: 20-40% and water: 3-4%) were also used as constraints for constructing central composite design space. The numerical and graphical optimizations were then run and the "*sweet spot*" corresponding to the most desirable formulation region compromising both responses were achieved.

Results: Phase diagrams showed a narrow area of existence of non-birefringent, transparent, viscoelastic region, which was extended as %PR incorporated into the system was increased. It was observed that as the lecithin concentration increased from 30 to 60 % w/w, drug incorporation capacity and viscosity increased while the flux of PR from organogels decreased remarkably. Also it was found through optimization that among the organogels investigated, those formulations containing 31.5-37.5 % w/w lecithin, 30.5-34.5 % w/w PR solutions and 3-3.35 % w/w water possessed the highest flux.

Major conclusion: Data confirmed that the choice of lecithin/IPM weight ratio and the amount of drug incorporated may be crucial in determining the performance of an organogel.

Keywords: Lecithin organogels, Propranolol hydrochloride, Release rate, Microemulsion-based gels, Modified Simplex, Central Composite

INTRODUCTION

Lecithin organogels (microemulsion-based gels; MBGs), first introduced by Scartazzini and Luici in 1988, are readily obtained by adding a minimal amount of a polar solvent (e.g. water) to a solution of lecithin in organic solvents (1). Lecithin is a natural mixture of phosphatides that account for more than 50% of the lipid matrix of biological membranes. The use of biocompatible, biodegradable, and non-immunogenic materials have made lecithin organogels suitable for long-term topical application (2-5). These systems are capable of solubilizing lipophilic, hydrophilic, and amphiphilic guest molecules, including enzymes. Thermodynamic stability, thermoreversibility in nature, insensitivity to moisture, resistant to microbial contamination, spontaneous formation and viscoelastic behavior are some of remarkable features of lecithin organogels (6, 7).

Experimental design and statistical analysis have been widely used to develop formulation as well as in process optimization and validation. The major advantage of experimental design for development of pharmaceutical products is that it allows all potential factors to be evaluated simultaneously and systematically. Using experimental design, one can evaluate the effect of each formulation factor on each response and possibly the effects of interaction between factors and, therefore, to identify the critical parameters based on statistical analysis. Once identified, the optimal formulation could be defined by using a proper experimental design to optimize the levels of all critical factors (8-16).

Response Surface Methodology (RSM) is a rapid technique used to derive a functional relationship between an experimental response and a set of

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input variables empirically. By using RSM, the number of experimental runs that is necessary for the establishment of a mathematical trend in the experimental design region will be reduced, allowing to determine the optimum level of experimental factors required for a given response (17-19).

The main aim of this research was to develop and optimize a semisolid preparation in an attempt to formulate a reservoir-type transdermal system with appropriate release profile. To achieve the final objectives, it seemed more reasonable to look for a vehicle that could interact with the skin and allow permeation of the drug into the skin. In this regard, the lecithin organogel was considered as potential and pharmaceutically acceptable vehicle for transdermal delivery. Propranolol as a beta blocker, mainly administered in the treatment of hypertension, was selected as the candidate drug to be incorporated in formulated lecithin gels. In the first step, the phase behavior of systems containing lecithin/isopropyl myristate (as the oil phase)/water/propranolol was investigated and in the second step, the data obtained was then used to optimize the propranolol release profile from the gels, using experimental design methods.

MATERIALS AND METHODS

Materials

Soybean lecithin (Epikuron 200; E200) and isopropyl myristate (IPM) were provided from Lucas Meyer Company (Germany) and Sigma Company (USA), respectively. Propranolol hydrochloride (PR) was gifted by Tolidaru Pharmaceutical Company (Iran). All other chemicals were obtained from Merck Co. (Germany). Purified water was prepared by a Millipore system (Millipore Corp., USA) and used for all experiments.

Methods

Construction of partial pseudo-ternary phase diagram

Samples containing different weight ratios of lecithin/IPM (20:80, 30:70, 40:60, 50:50 and 60:40) were initially prepared. Phase studies were carried out by adding either pure water or solutions of PR with various concentrations (10, 20, 30, 50% w/v) to the mixture of lecithin/IPM, while stirring. After addition of each 10 µl aqueous solution, resulting systems were examined for clarity and viscosity. The endpoint of the organogel domain at a given ratio was determined when the system became turbid after addition of a specific amount of aqueous phase. The phase behavior of the system was mapped on phase diagrams with the top apex representing IPM and the other apices representing lecithin and water or PR solution. The transparent, homogenous, non-birefringent, isotropic area enclosed by the line connecting the endpoints was considered as the microemulsion-based organogel domain.

Construction of calibration curves

Three series of different concentrations (i.e. 5, 10, 15, 25, 50, and 75 µg/ml) of PR in HCl medium (1% v/v) were prepared to construct the calibration curves. Solutions were analyzed spectrophotometrically at the wavelength of 289 nm.

Release studies

Cellulose acetate membrane (MWCO 3500) was soaked in distilled water for 12 hrs. The release of PR from the lecithin organogels through the selected membrane was investigated using Franz–diffusion cells, having a diameter of 9 mm and a receptor volume of 5.1 ml. The artificial membrane was placed between the donor and receptor compartments of the cells. The effective area of membrane available for diffusion was 0.64 cm^2. In all experiments, 0.35 g of each drug-containing formulation was placed over the membrane, and the donor department was then covered with parafilm. The receptor compartment was filled with 5.1 ml of degassed 1% HCl solution. The cells were thermostated at 32°C in an incubator, and the receptor solution was stirred with a magnetic stirrer at 500 rpm throughout the experiment. The receptor phase was withdrawn at predetermined intervals up to 9 hrs and replaced by fresh 1% HCl solution equilibrated at 32°C. Drug concentration was determined using a spectrophotometer Cecil CE 2021 UV at λ=289 nm.

Viscosity measurements

Viscosity of each sample was measured, using a cone & plate Brookfield viscometer at a controlled temperature of 25°C.

Modified simplex

Modified Simplex was used for the sequential design of experiments and optimization. In this study, three factors (k), including lecithin, PR and water were selected. IPM, as another factor, was considered as 100- % lecithin. In the first step, we started with four (k+1) runs and moved forward to the desired responses through measuring various projections of the rejected trial condition (W), Reflection (R), Expansion (E), CR or C^+(positive contraction) and CW or C^- (negative contraction). The narrower region of these factors corresponding to near optimum conditions was further used to construct RSM design.

RSM (Central Composite) design

The experimental data was analyzed by response surface regression procedure and the results were statistically analyzed by the corresponding analysis of variances of the selected experimental design. If three factors are studied at two levels, the relevant equation would be equation 1, with three two-way

Table 1. Independent variables and their constraints.

Independent variables	Unit	Lower constraint	Upper constraint
lecithin*	%	30	50
PR	%	20	40
water	%	3	4

interactions and one three-way interaction (9).

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3+\varepsilon \quad \text{(equation 1)}$$

If a quadratic relationship is sought, then equation (2) would be used (9).

$$Y=\beta_0+\beta_1X_1+\beta_{11}X_1^2+\beta_2X_2+\beta_{22}X_2^2+\beta_3X_3+\beta_{33}X_3^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3+\varepsilon \quad \text{(equation 2)}$$

The cumulative amount of the drug permeation through synthetic membrane was plotted as a function of time and linear regression analysis was then used to calculate the penetration rate (flux) of drug. The flux (response 1), viscosity (response 2) and formulation variables of all model formulations were treated by Design-Expert® software (version 7.0.0, State ease Inc, Minneapolis, USA). A suitable polynomial model were selected based on the significant terms ($p < 0.05$), the least significant lack of fit, coefficient of variation (CV), the multiple correlation coefficient (R^2), and adjusted multiple correlation coefficient (adjusted R^2) provided by Design-Expert® software. The default model in the design of the experimental space was quadratic and the upper and lower levels are demonstrated in table 1.

Optimization

Two responses of flux and viscosity were selected for both numerical and graphical optimization. Since the inverse relation between flux and viscosity in organogels is well established, it was decided to choose maximization for flux and a minimization for viscosity in order to obtain an optimized formulation. On a contour plot, we should visually search for the best compromise which stands for the formulation with desirable values for both responses, simultaneously.

Verification

Five provided optimized formulations were prepared experimentally and both responses (i.e. viscosity and flux) were determined.

RESULTS AND DISCUSSION

Partial phase diagram

Partial phase diagrams of systems containing lecithin/IPM/water or various PR solutions at five different lecithin/IPM weight ratios were constructed. Figure 1 illustrates one typical phase diagram constructed in the presence of water at lecithin/IPM weight ratio of 60:40. Similar trends were obtained when water was replaced with PR solutions and surfactant/oil ratio was changed (diagrams are not presented). It should be noted that in this study systems containing more than 60 % lecithin was not evaluated, due to their high viscosity. As it can found in figure 1, irrespective of the lecithin/IPM ratio, the isotropic, transparent region extends over a narrow area and covers the whole range of surfactant concentrations which were investigated in this study. It is clear that % PR exerts a significant effect on the extent of the organogel region. In general, following generalizations for the systems studied can be made.

1) in all systems, the MBG region extended over a very narrow area in the water poor part of the phase diagram,
2) for a given system, as lecithin/IPM ratio increased, the aqueous phase incorporation capacity was also increased,
3) regardless of the surfactant/oil ratio, the capacity for the incorporation of PR solutions was more than that of water,
4) A decrease in viscosity, cloudiness and formation of two-phase systems were observed when water was in excess.

Construction of phase diagrams can determine the extent of organogel domain. A decrease in viscosity and the appearance of cloudiness (probably due to the presence of a two-phase system) are observed when water was in excess. It should be noted that dissolving of PR in lecithin/IPM solution, followed by addition of water leads to the formation of a turbid system. Therefore, in this study, it was decided to incorporate PR solution into the lecithin/

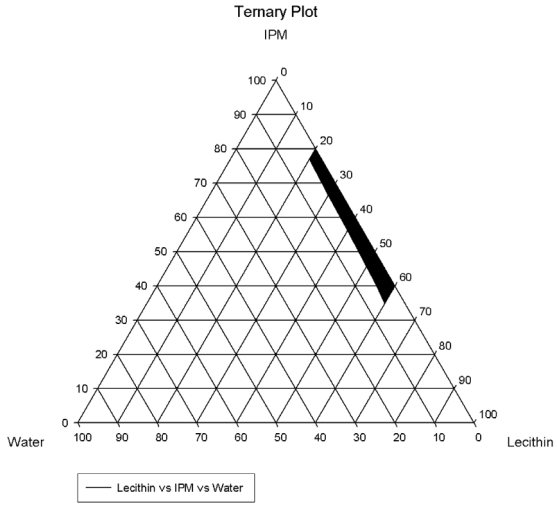

Figure 1. Partial phase diagram of organogels containing lecithin/IPM and water at lecithin/IPM weight ratio of 60:40.

Table 2. Experimental CCD matrix: design points and selected responses.

				Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	**Run**	**Point Type**	**Block**	**A:lecithin**	**B:PR**	**C:Water**	**Flux**	**Viscosity**
				%	**%**	**%**	**microgram/cm²/hr**	**p**
1	7	Fact	Block 1	50	40	3	360	560
2	4	Fact	Block 1	50	30	4	350	510
3	1	Fact	Block 1	30	40	4	500	460
4	5	Fact	Block 1	30	30	3	505	366
5	3	Center	Block 1	40	35	3.5	469	402
6	6	Center	Block 1	40	35	3.5	490	377
7	2	Center	Block 1	40	35	3.5	470	385
8	8	Axial	Block 2	25	35	3.5	479	375
9	16	Axial	Block 2	54	35	3.5	360	620
10	13	Axial	Block 2	40	28	3.5	440	379
11	11	Axial	Block 2	40	42	3.5	380	419
12	9	Axial	Block 2	40	35	2.8	487	380
13	10	Axial	Block 2	40	35	4.2	400	397
14	15	Center	Block 2	40	35	3.5	483	376
15	14	Center	Block 2	40	35	3.5	478	395
16	12	Center	Block 2	40	35	3.5	486	370

IPM mixture.

For the preparation of lecithin organogels, a sharp increase in viscosity is observed at water to lecithin molar ratio, n_w, of 3 (20**).** The organogel state is maintained up to a particular molar ratio, designated as n_{cr}. At the state in which n_w is equal to n_{cr}, the maximum viscosity of organogel is achieved. By the addition of water above the n_{cr} (i.e. at $n_w > n_{cr}$), the three-dimensional network collapses and separation of the homogenous organogel takes place via a two-phase system, consisting of a low viscous liquid and a compact organogel or jelly-like phase. For lecithin organogels which were prepared by using IPM, this stage is observed at n_w of 5-6 (20). It has also been shown that as the lecithin/IPM ratio increases, the gel solubilizing capacity increases. This could be attributed either to the increase in the number of cylindrical micelles or to the further growth of cylindrical micelles or both. However, since the amount of water required to obtain the gel is very low and the gel formation is a function of the molar water to lecithin ratio of about 3, organogel systems in general show potentially low capacity for guest molecules (21).

Viscosity and flux measurements

Viscosity of the formulations investigated in this study were found to be in the range of 350-650 Poise. It was observed that an increase in the amount of incorporated water or PR solution and also in the lecithin/IPM ratio resulted in an increase in the viscosity. The drug release rate (flux), calculated by using the calibration curve with a coefficient of 0.9997, ranged from 350-500 μg/cm²/hr, in the way that the lowest flux was observed in the most viscous formulation.

Modified Simplex and Central Composite Design (CCD)

Modified Simplex method was applied in order to reach to a nearly desirable region through Central Composite Design (17, 18). The proposed constraints were lecithin 30-50 %, PR 20-40 % and water 3-4%. Central composite design is an experimental design, useful in response surface methodology, for building a second order (quadratic) model for the response variable (19). The design consists of three distinct sets of experimental runs, including a factorial design, a set of *center points*, and a set of *axial points*. We chose a rotatable CCD with $\alpha = 1.68$, compromising three factors at two levels, with randomized order, 2 blocks , 6 center points and 10 non-center points (i.e. 4 factorial and 6 axial points). Flux and viscosity were assigned as dependent variables to be optimized. Table 2 summarizes the experimental design runs and results obtained for two responses. It should be noted that % IPM was considered as100- % lecithin.

Statistical analysis of data

In the RSM analysis, the responses (i.e. flux and viscosity of all model formulations) were treated

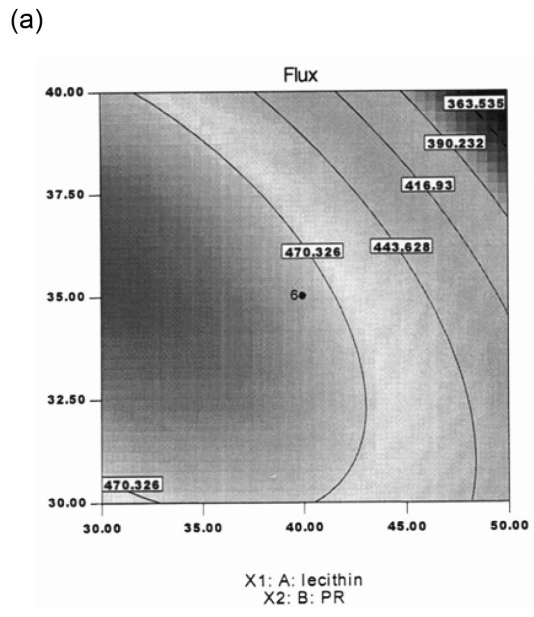

Figure 2. Plots of flux versus X_1: lecithin, X_2: PR at 3.5% water as the actual factor; *a*) contour plot; *b*) 3D plot.

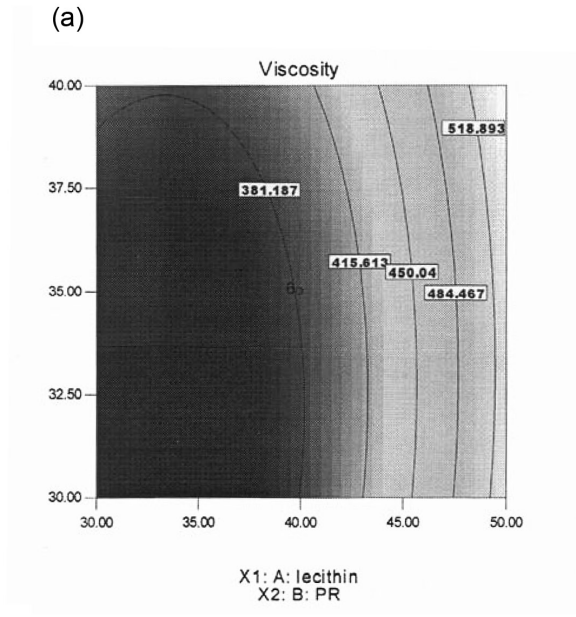

Figure 3. Plots of viscosity versus X_1: lecithin, X_2: PR at 3.5% water as the actual factor; *a*) contour plot; *b*) 3D plot.

by Design-Expert® software. The best fitting mathematical model was selected based on the comparisons of several statistical parameters, including the coefficient of variation (CV), multiple correlation coefficient (R^2) and adjusted multiple correlation coefficient (adjusted R^2). Analysis for both responses showed that quadratic model was the most suitable one *($p < 0.05$)*. The statistical analysis proved that A, B, C, BC, A^2, B^2 are significant model terms for response 1 (flux) and A, BC, A^2, B^2 are significant model terms for response 2 (viscosity). Contour plots and 3D graphs of both responses are demonstrated in Figures 2 and 3.

By runnig ANOVA, the final equation of flux in coded values was obtained (equation 3), while the statistical parameters were as follows:

CV = % 3.51 , R^2 = 0.9738, adjusted R^2 = 0.9265

$$Flux = 477.41 - 42.07\,A - 21.21\,B - 30.76\,C - 27.01\,AB - 22.46\,AC + 31.68\,BC - 22.76\,A^2 - 27.51\,B^2 - 10.76\,C^2 \quad \text{(equation 3)}$$

As stated before A, B, C, BC, A^2, B^2 are significant model terms for response 1 (flux). It is concluded that the flux has negative relationship with lecithin, PR and water content as the main effect. The model also introduced A^2, B^2 as significant negative interactions and BC as positive interaction effect on this response.

In the same way, the final equation of viscosity in

coded values was obtained (equation 4), while the statistical parameters were as follows:

CV = % 2.95, R^2 = 0.9907, adjusted R^2 = 0.9739 .

Viscosity = 383.48 + 86.62 A + 14.14B + 6.0 C - 4.99 AB - 21.86 AC + 25.62 BC + 61.66A^2 + 12.41B^2 + 7.16 C^2 (equation 4)

As observed, A, BC, A^2 and B^2 are significant model terms for response 2 (viscosity). It seems that viscosity has positive relationship with lecithin as the main effect. The model also introduced BC as posistive interaction effect on this response.

Mathematical relationship generated for the studied response variables were expressed as equations 3 and 4, showing the coefficients for intercept, first and second order effects and interaction terms. The aim of optimization was to obtain the defined targets for both responses simultaneously with respect to

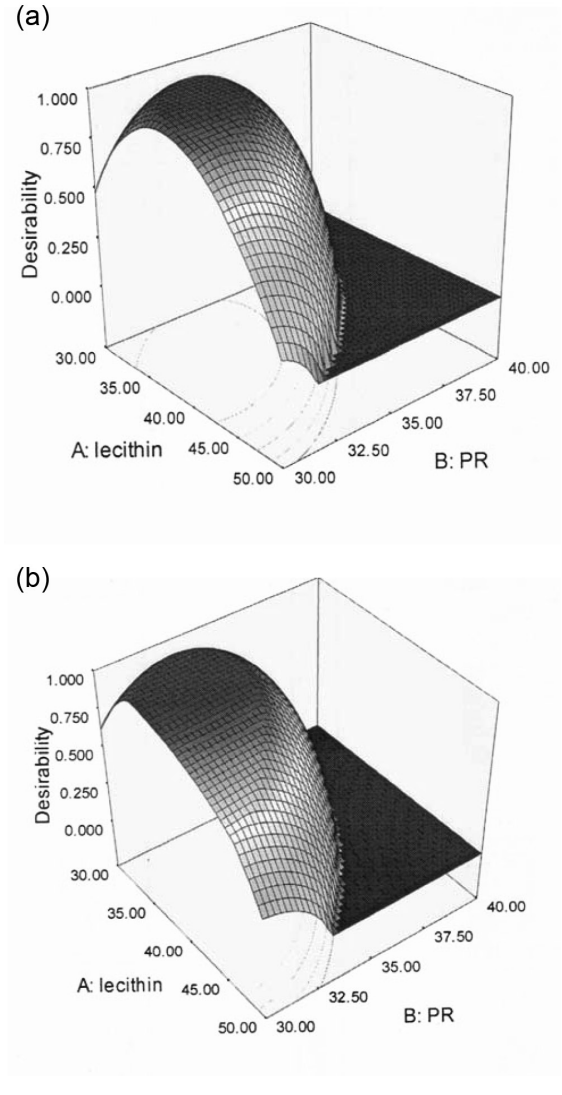

Figure 4. 3D plots of the predicted formulations (X_1: Lecithin, X_2: Pr) at *a*) 3.2% water content (C: Water = 3.20) ; *b*) 3.1% water content (C: Water = 3.10).

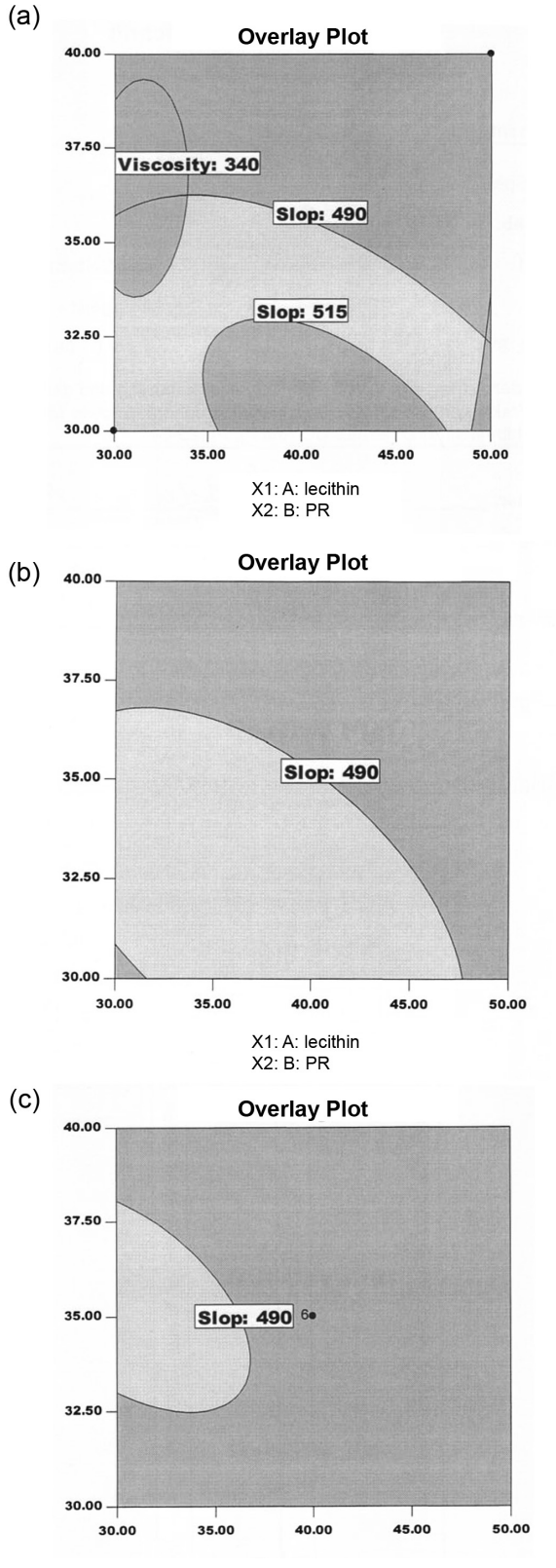

Figure 5. Overlay plots of responses (flux & viscosity) for predicted formulations at various water content as the actual factor (X_1: Lecithin, X_2: Pr); *a*) 3.1% (C: Water = 3.00); *b*) 3.2% (C: Water = 3.20) and *c*) 3.5% (C: Water = 3.50). The light gray region stands for formulations with maximum flux and minimum viscosity.

Table 3. Experimentally prepared formulations based on the predicted results and the evaluation of flux and viscosity.

	Components			Flux (μg/cm²/h)		Viscosity (p)		
Number	**Lecithin (%)**	**PR (%)**	**Water (%)**	**Predicted**	**Observed**	**Predicted**	**Observed**	**Euclidean distance**
1	34.69	31.76	3.03	514	510	354	350	5.6
2	25.2	30.77	3.15	507	503	358	361	5
3	36.35	32.53	3.21	507	505	356	358	2.8
4	35.16	31.64	3.05	513	510	356	348	8.5
5	36.94	33.12	3.1	510	508	360	353	7.3

the predefined constraints. In this study, flux was set to be maximized while the viscosity was set to be minimized. The final stage was to overlap the defined desirable areas of both responses to generate the region of interest or *sweet plot*. Figure 4 demonstrates the 3D plots of one of the predicted formulations at the 3.2% and 3.1% water contents, selected from the optimum formulations with predicted responses (data is not presented). Overlay plots of responses (flux & viscosity) for predicted formulations at three different water contents as the actual factor are depicted in figure 5. The yellow region stands for formulations with maximum flux and minimum viscosity.

In order to confirm the desirability of provided optimized formulations, five formulations were prepared experimentally and the two responses of flux and viscosity were evaluated (Table 3). It was observed that the experimentally obtained and the predicted responses were closely related and therefore, the optimization process was verified. This was confirmed by calculating the Euclidean distance (Ed),using the following equation (22):

$$Ed = (\sum_i (Pred._i - Obs._i)^2)^{1/2}$$

where $Pred._i$ and $Obs._i$ are predicted and observed values, on response *i* respectively and the summation was overall responses. Minimized Euclidean distance is an important factor to demonstrate closeness between predicted and observed responses.

In conclusion, organogels containing 31.5-37.5% w/w lecithin, 30.5-34.5 % w/w PR and 3-3.35 % w/w water showed the highest flux, and the most optimized system which was predicted by the application of experiment design methods was composed of 36.35% lecithin, 32.53% PR and 3.21% water (the system with the minimum difference between the predicted and observed responses).

CONCLUSION

The main objective of the present study was to prepare a lecithin-based microemulasion gel (organogel) containing propranolol HCl with a predictable release rate. Data confirms that the choice of lecithin/IPM weight ratio and the amount of drug incorporated may be crucial in determining the performance of a microemulaion-based gel. Experimental design methods were used to optimize the release of PR from the gel. The tested parameters were lecithin, PR and water contents and the gel viscosity and flux were considered as responses for optimization. Our study demonstrates that experimental design technique is a valuable tool for optimization of organogel formulations, which enables to have a better understanding of how different, crucial variables could influence the selected responses.

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