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Research Article

Statistical Optimization and Characterization of Nimodipine Transferosomes

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Abstract

Background: Nimodipine is a vasodilator that is used for the prevention of cerebral vasospasm after subarachnoid hemorrhage. The oral and intravenous administration of the drug is associated with undesirable side effects. So, transdermal delivery using lipid-based nanovesicles, also known as transferosomes, can be thought of as an alternative. ***Objective***: To optimize the formulation of transferosomes using the statistical design of experiments, with the aim of obtaining the most suitable transferosomes for the transdermal delivery of nimodipine. ***Methods***: In the Box-Behenken statistical design, the independent variables were the quantities of nimodipine, phospholipon 90%, and sodium deoxycholate, while the dependent variables were the vesicle size, entrapment efficiency for nimodipine and its flux through the rat's skin. The optimized formulation was characterized through transmission electron microscopy and the deformability index. ***Results***: The optimized formulation of transferosomes suggested by the software consisted of 30 mg nimodipine, 150 mg phospholipon 90% and 15 mg sodium deoxycholate. The resulted values were 248 nm for vesicles size, 81% for entrapment, and 476 µg/cm^2/h. Under transmission electron microscopy, transferosomes appeared as vesicles, with a 0.98 deformability index for the optimized formula. ***Conclusions***: Nimodipine can be formulated as transferosomes and efficiently applied for transdermal delivery.

Keywords: Box-Behenken design, Flux, Nimodipine, Transferosomes.

التحسين الإحصائي والتوصيف للحويصلات الناقلة الشحمية للنيموديبين

الخلاصة

الخلفية: نيموديبين هو دواء موسع للأوعية الدموية يستخدم للوقاية من تشنج الأوعية الدماغية بعد النزيف تحت العنكبوتية. يؤدي أخذ الدواء عن طريق الفم أوالوريد إلى آثار جانبية غير مرغوب فيها ، لذلك يمكن إعتبار التوصيل عبر الجلد خيار بديل من خلال صياغة الحويصلات الناقلة النانوية ذات الأساس الدهني. **الهدف**: تحسين صياغة الحويصلات الناقلة من خلال تطبيق التصميم الإحصائي للتجارب و ذلك للوصول إلى أنسب مواصفات تلك الحويصلات لتكون قادرة على توصيل نيموديبين عبر الجلد. **الطرق**: عند تطبيق التصميم الإحصائي بوكس-بينكن، كانت المتغيرات المستقلة هي كمية نيموديبين، وفوسفوليبون 90% ، و ديوكسيتشولات الصوديوم ، بينما كانت المتغيرات غير المستقلة كل من حجم الحويصلات وكفاءة الانحباس للنيموديبين وتدفقه من خلال جلد الجرذان. تم إجراء توصيفات إضافية للصيغة الأمثل بإستخدام المجهر الإلكتروني النافذ ومؤشر التشوه. **النتائج**: وجد أن الصيغة الأفضل للحويصلات الناقلة المقترحة من التطبيق الإحصائي تتكون من 30 ملجم نيموديبين و150 ملجم فوسفوليبون 90% و 15 ملجم صوديوم ديوكسيكولات. وكانت القيم الناتجة 248 نانومتر لحجم الحويصلات، و 81٪ لكفاءة تحميل الدواء، والنفاذ عبر الجلد 476 ميكروجرام/سم2/ساعة. أظهرت صور المجهر الإلكتروني أن الحويصلات الناقلة لها غشاء ثنائي الطبقة و إن قيمة مؤشر التشوه هي 0.98 لهذه الصيغة. **الاستنتاجات**: أظهرت الدراسة إمكانية صياغة نيموديبين على شكل حويصلات ناقلة لتطبيقها في التوصيل عبر الجلد.

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INTRODUCTION

Effective delivery systems designed for local, systemic, or targeted delivery enable drugs to achieve the desired therapeutic effect [1]. Transdermal drug delivery combines the advantages of non-invasiveness and suitability for unconscious patients with a minimum incidence of side effects [2]. Nevertheless, in order to reach systemic circulation, the delivery of drugs must overcome the multiple and complex layers of the skin [3]. Lipid-based systems enhance the bioavailability of drugs significantly [4]. More specifically, such nano-scaled systems gained attention due to their lipid constituents, which resemble those of the skin [5]. Researchers developed several types of lipid carriers,

such as liposomes [6], transferosomes [7], ethosomes [8], invasomes [9], novasomes [10], and cubosomes [11], which marked a breakthrough in transdermal drug delivery. Liposomes, however, deposit in the stratum corneum, so their effectiveness in transdermal delivery is limited [12]. To overcome this drawback, surfactants replaced cholesterol in transferosomes, resulting in an ultra-deformable and highly flexible structure [13]. Transferosomes consist of phospholipid, an edge activator, ethanol, and water [14]. The most commonly used phospholipids are phosphatidylcholine esters. The edge activator is a surfactant or bile salt that imparts deformability and flexibility so that transferosomes can penetrate the skin [15]. The edge activators include sodium cholate, sodium deoxycholate, Spans, and Tweens [16]. Subarachnoid hemorrhage is a type of cerebrovascular disorder that can be detected and evaluated by computed tomographic angiography [17]. Nimodipine is a calcium channel-blocking drug that acts as a selective vasodilator for the prevention of cerebral vasospasm after subarachnoid hemorrhage [18]. The oral and parenteral products of the drug are associated with hypotension as the main side effect [19]. Furthermore, the short half-life of the drug due to extensive first-pass metabolism requires multiple administrations or infusions through the central vein. [20]. Therefore, transdermal delivery of nimodipine can help minimize fluctuations in the drug's plasma level, thereby reducing adverse events, particularly hypotension, and reducing the frequency of dosing, ultimately improving patient compliance [21]. The objective of this study is to formulate nimodipine as transferosomes through optimization of the quantities of nimodipine, phospholipon 90%, and sodium deoxycholate by statistical design of experiments. Further characterization of the optimized formulation aims to evaluate its application for transdermal drug delivery.

METHODS

Materials and instruments

Nimodipine was purchased from Leyan Co. (China) and phospholipon 90% from Henan Co. (China). The sodium deoxycholate supplier was BDH (UK). Chloroform and ethanol were purchased from Supelco (UK). Monobasic potassium phosphate, sodium hydroxide and sodium lauryl sulfate (Alpha, India). The instruments that were used in this work include a rotary evaporator (Buchi, Switzerland), an ultrasound water bath (Soniclean, Australia), a zetasizer (Malvern, USA), a centrifuge (Eppendorf, Germany), a UV spectrophotometer (Shimadzu, Japan), a Franz diffusion apparatus (Copley, UK) and a transmission electron microscope (Zeiss, Germany).

Experimental design

This study applied a Box-Behenken statistical design. Design Expert Software Version 13 (Stat-Ease Inc., Minneapolis, MN) generated 15-run designs, each variable with three levels. The selected independent variables include the quantity of nimodipine (A), the quantity of phospholipon 90% (B) and the quantity of sodium deoxycholate (C). The dependent variables (responses) were vesicle size, entrapment efficiency, and flux (permeation) of the drug. Table 1 contains the formulations for the 15 runs suggested by the software.

Table 1: The independent variables and the responses obtained for the formulation trials

Run	(A) Nimodipine (mg)	(B) Phospholipon 90% (mg)	(C) Sodium deoxycholate (mg)	VS (nm)	EE (%)	Flux (μg/cm^2/h)
1	45	200	20	345	82.5	469.3
2	30	200	15	330	84.3	337.1
3	30	150	10	225	77.4	243.8
4	15	200	20	349	83.3	462.3
5	45	150	15	247	81	379.4
6	15	150	15	201	74.7	475.2
7	15	100	20	99	65.3	432.3
8	30	100	15	104	63.4	443.7
9	30	150	15	196	76.9	410.4
10	30	150	20	182	72.6	433.5
11	45	100	10	104	66.4	219.9
12	45	200	10	328	82.1	274.1
13	30	150	15	225	78.1	257.8
14	30	150	15	216	74.5	270.3
15	15	100	10	98	69	235.6

Transferosomes preparation

Thin film hydration after solvent evaporation was applied for the preparation of nimodipine-loaded transferosomes. The method was started by dissolving nimodipine, phospholipon 90% and sodium deoxycholate in a chloroform and methanol mixture (3:1). The resultant solution was transferred to a round flask for evaporation using the rotary evaporator, which was set at a speed of 80 rpm, a water bath temperature of 55°C and reduced pressure. Afterward, the formed film on the flask wall was left for two hours to complete dryness. The film was hydrated with 20 mL of phosphate buffer, pH 7.4 and slight shaking for two hours at a temperature of 70 °C. Finally, the dispersion was sonicated for 3 minutes in an ultrasound water bath [22].

Characterization of the formula

The technique of dynamic light scattering was applied by the zetasizer instrument to determine the size of the vesicles. Prior to analysis, we diluted the transferosome dispersions with water [23]. Testing the entrapment efficiency of nimodipine in transferosomes was started with centrifuging aliquots from the transferosome dispersion in Amicon® tubes at 14000 rpm for 30 minutes [29]. Nimodipine in the filtered and retained portions (free and total, respectively) was quantified by measurement of UV absorbance at 238 nm through a 1 cm cuvette in the spectrophotometer after suitable dilution of the samples. The entrapment efficiency of nimodipine was determined using the following equation [24]:

$$EE\ (\%) = (\text{Total nimodipine} - \text{Free nimodipine})/(\text{Total nimodipine}) \times 100$$

The ex vivo steady-state flux was determined utilizing a modified Franz diffusion cell with an area of 3.14 cm^2. An excised skin of Wister rat males was used after the removal of hair and subcutaneous tissues [25]. The washed rat's skin was placed between the donor and receiver chambers of the cell. Phosphate buffer pH 6.8 with 1% sodium lauryl sulfate was filled in the receiver chamber at a temperature of 35±0.5 ºC, while the transferosome dispersion was placed in the donor chamber. Aliquots from the receiver medium were withdrawn at 1, 2, 4, 6, 12, 16, and 24 hours. After each sampling, the volume was replaced [26]. The permeated quantities of nimodipine were obtained from the UV absorbance at a maximum wavelength of 238 nm through a 1-cm cuvette utilized in the spectrophotometer after suitable dilution of the samples [27]. The steady flux (Jss) of nimodipine was calculated from the linear part of the plotted quantities versus time for each experimental run.

Optimization of transferosomes

We prepared the optimized formulation using the suggested quantities of nimodipine, phospholipon 90%, and sodium deoxycholate, following the previously described method. We then characterized the vesicles size, entrapment efficiency, and nimodipine flux for the 15-run formulation to compare the predicted and observed values [28]. Imaging with transmission electron microscopy (TEM) was performed in order to examine the structure of the optimized nimodipine-loaded transfersomes. A mini-drop of the transferosome dispersion was dried on a metallic grid and then subjected to imaging [29]. The deformability index is an important parameter because it reflects the ability of transferosome vesicles to traverse through skin layers with retained integrity. Extrusion was the technique used for the measurement of the deformability of an optimized formulation. A vacuum pump was connected to a glass flask, and a membrane with a pore size of 0.20 µm was fitted to a stainless-steel holder at the top. As the pump switched on, the dispersion formulation passed through the membrane. The sizes of vesicles were measured before and after extrusion using a zetasizer [30]. The deformability index was calculated by dividing the measured sizes after extrusion by those recorded before this process.

RESULTS

Table 1 displays the vesicle size, entrapment efficiency, and steady-state flux of nimodipine for the 15 runs of formulations, while Table 2 summarizes the statistical parameters obtained for the responses. Linear models were suggested for the three responses, and their F values indicated that these models were significant.

Table 2: Statistical parameters obtained for various responses for the linear models

Response	Model F-value	Significant terms*	Lack of fit F-value	Predicted R2	Adjusted R2	Adequate precision
Vesicles size	319.69	B	1.37	0.9502	0.9653	28.1244
Entrapment efficiency	30.75	B	2.10	0.8057	0.9740	15.1481
Steady-state flux	6.86	C	0.4842	0.4761	0.5567	6.7521

*A is the quantity of nimodipine, B is the quantity of phospholipon 90%, and C is the quantity of sodium deoxycholate.

There are no significant interactions between the quantity of nimodipine, the quantity of phospholipon 90%, and the quantity of sodium deoxycholate. The predicted and adjusted R^2 values were found to be reasonable closeness as well as adequate precision values for all three responses that complied with the intended models. For vesicle size (VS), we proposed the following polynomial linear model:

$$VS = 216.1 + 5.2A + 117.2B - 1C$$

The model indicated that the quantity of phospholipon 90% that specifically had the greatest influence on the size of the vesicles compared to the other two variables. Using a larger quantity of phospholipon (90%) results in increased vesicle size. In contrast, a larger quantity of sodium deoxycholate appeared to reduce the size of the vesicles, but this effect was at its minimum. As observed in the contour and response surface plots (Figures 1 and 2), the quantity of phospholipon 90% very slightly increased when the quantity of nimodipine decreased.

Thus, at higher quantities of phospholipon 90%, the vesicles size enlarged regardless of the change in the quantity of sodium deoxycholate. The resulting linear model was a polynomial equation for entrapment efficiency:

$$EE = 74.33 - 0.01A + 8.79B - 2.2C$$

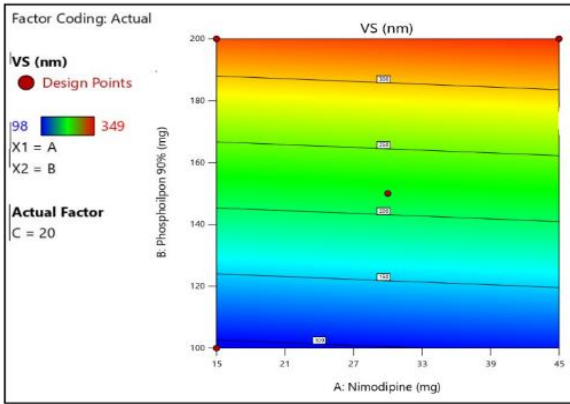

Figure 1: Contour plot for the effect of independent factors on the vesicles (VS).

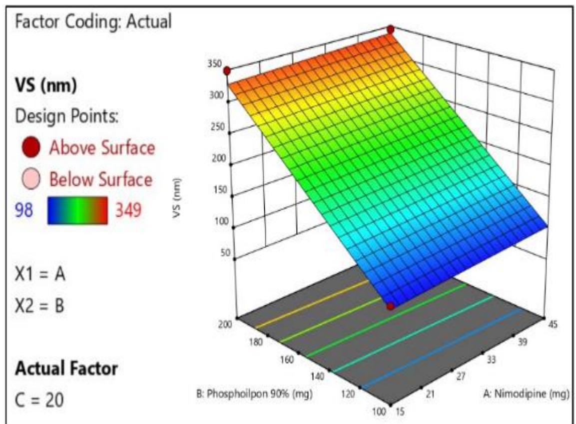

Figure 2: Response surface plot for the effect of independent factors on the vesicles size (VS).

As the model's coefficients indicated, the quantity of phospholipon 90% appeared to be the most influential direct parameter on entrapment efficiency, and the effect of the quantity of nimodipine was negligible. On the other hand, sodium deoxycholate had the opposite effect. The contour and response surface plots for entrapment efficiency in Figures 3 and 4 revealed the linearity of the response. We created a linear model as the polynomial equation to describe the steady-state flux:

$$Jss = 456.21 - 8.77A + 3.63B + 199.8C$$

The quantity of sodium deoxycholate is a directly dominant parameter that affects the steady-state flux. The quantity of phospholipon 90% had a similar effect, albeit to a lesser extent, while the quantity of nimodipine showed a paradoxical response. Increasing the edge activator in this study, sodium deoxycholate, leads to a greater penetration of nimodipine. The quantity of nimodipine had a negative effect here, so the flux decreased at higher quantities of the drug. In Figures 5 and 6, the contour and response surface plots for steady-state flux showed a liner-positive correlation. We chose 30 mg of nimodipine, 150 mg of phospholipon 90%, and 15 mg of sodium deoxycholate for the formulation's numerical optimization. The software suggested a solution of 30 mg, 170 mg, and 20 mg for the nimodipine-loaded transferosomes, respectively, to optimize the formulation.

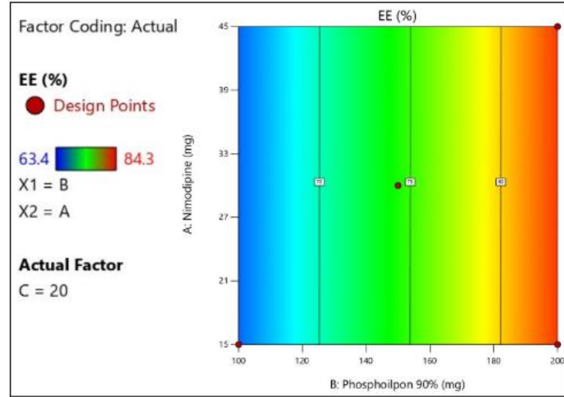

Figure 3: Contour plot for the effect of independent factors on the entrapment efficiency (EE).

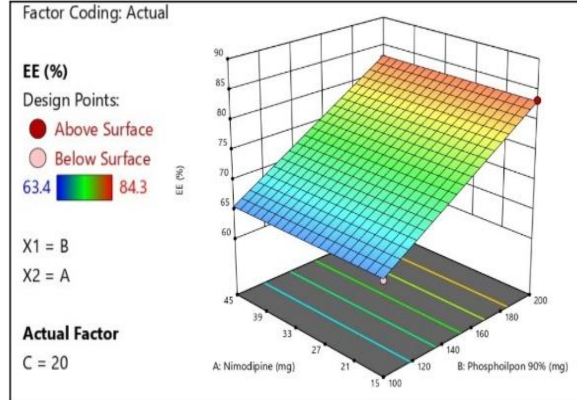

Figure 4: Response surface plot for the effect of independent factors on the entrapment efficiency (EE).

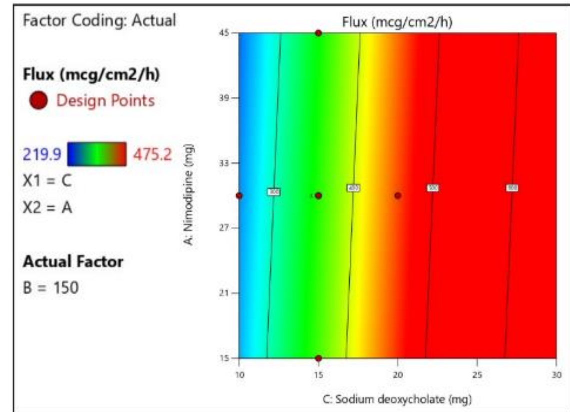

Figure 5: Contour plot for the effect of independent factors on the steady-state flux (Jss).

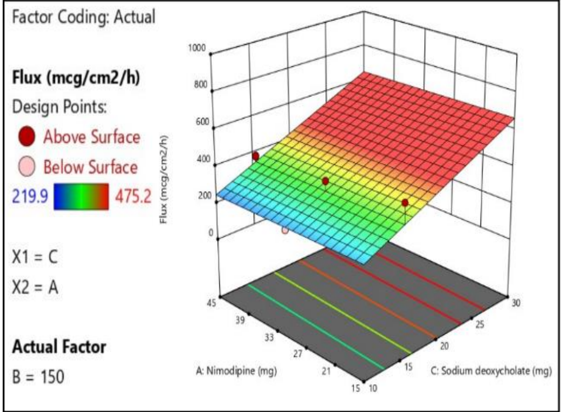

Figure 6: Response surface plot for the effect of independent factors on the steady-state flux (Jss).

The optimized formulation was prepared and characterized. Table 3 illustrates the good agreement between the predicted and observed values.

Table 3: Responses for the optimized formulation

Response	Predicted value	Observed value
Vesicles size (nm)	263.5	248
Entrapment efficiency (%)	77.9	81
Steady-state flux	457.68	476

The image obtained by TEM in Figure 7 revealed the vesicular, bilayer structure of the optimized transferosomes. In addition, the size of an individual transferosome was approximately similar to that measured by zetasizer. Finally, we calculated the deformability index for the optimized formulation and found it to be 0.98, reflecting the elastic property of the transferosomes, enabling them to reach the deeper skin layers with acceptable deformability.

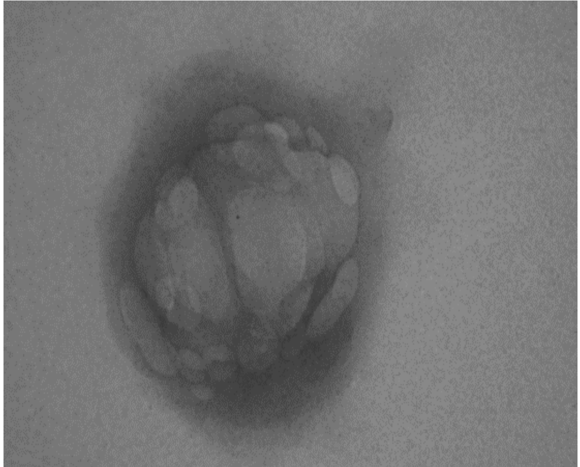

Figure 7: Transmission Electron Microscope image of nimodipine-loaded transferosomes.

DISCUSSION

Choosing the right models during experimental design is critical for any formulation and/or process development. In this study, the linear models showed that the amounts of nimodipine, phospholipon 90%, and sodium deoxycholate all had their own effects on the results. On the other hand, the calculated statistical parameters for the responses, specifically the close similarity between predicted and adjusted R^2 values, indicated that responses can be predicted and the explanatory power of the models for different numbers of terms [31]. Also, the high values (more than 4) for adequate precision showed that the signal-to-noise ratio was good enough. This meant that these linear models were good enough to describe the relationship between the independent and dependent variables, which made the formulation work better. While the quantity of surfactant edge activator inversely affects the size of the vesicles [32], the type of edge activator only slightly influences the vesicles' size [33]. Additionally, stabilizing the elastic membrane attributed to the surfactant can prevent aggregation and subsequent size increments [34]. On the other hand, the quantity of nimodipine did not influence the vesicle size, although previous studies reported that a higher ratio of the drug to lipid would increase the vesicle size [35]. Entrapment efficiency in some studies did not show a correlation with the edge activator [36]. However, a larger quantity of edge activators also exhibited higher drug entrapment [37,38]. The chosen type and quantity of the edge activator might explain the diversity of these results. The extreme membrane fluidity of transferosomes, which in turn could lead to the formation of pores and eventual leakage of the incorporated drug, may account for the unexpected results of this study [39]. The above model revealed a positive correlation between the quantity of phospholipon 90% and the entrapment efficiency, bolstered by the lipophilic property of nimodipine. This suggests that a larger quantity of phospholipon 90% could slightly stiffen the vesicular membrane [40]. Larger quantities of lipid could have countered the edge activator's effect, as the contour and response surface plots swiftly revealed. In the context of steady-state flux, the observed perpendicular trait, attributed to the quantity of sodium deoxycholate, demonstrated a significant enhancement of flux. These results agreed with those published for other drugs [24]. The fact that edge activators not only maintain the flexibility and elasticity of the vesicles but also function as surfactants to improve drug permeation elucidates this characteristic effect [30]. The low permeability of liposomes reveals the significantly lower effect of phospholipon 90%, which restricts its intended use for topical delivery in comparison to transferosomes that incorporate edge activators for improved transdermal delivery [41]. This study found a negative correlation between the quantity of nimodipine and flux, potentially due to the lipophilic nature of one arm and the high drug quantities in the other. Therefore, this saturation state of nimodipine decreased its hemodynamic activity and subsequently reduced its flux [42]. The software's selection of desirability for optimization and its suggested formulation produced comparable responses for both the predicted and observed values. TEM imaging and the deformability index further validated this, as they both demonstrated the transferosomes' capacity to navigate the skin's layers while maintaining their integrity, thereby delivering nimodipine.

Conclusion

Transferosomes can be considered potential carriers for nimodipine due to their ability to enhance skin permeation in a controlled manner. These advantages provide convenient administration for patients as well as a reduction in the side effects associated with nimodipine.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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