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## ORIGINAL ARTICLE

# Improved bioavailability of timolol maleate via transdermal transfersomal gel: Statistical optimization, characterization, and pharmacokinetic assessment

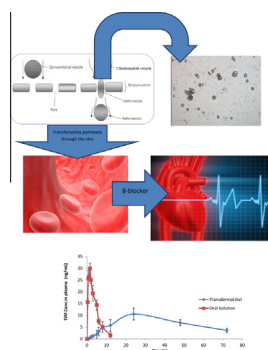


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## GRAPHICAL ABSTRACT



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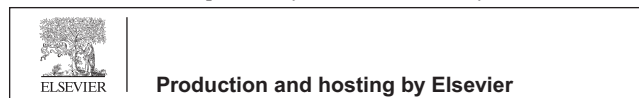
## ABSTRACT

Timolol maleate (TiM), a nonselective  $\beta$ -adrenergic blocker, is a potent highly effective agent for management of hypertension. The drug suffers from extensive first pass effect, resulting in a reduction of oral bioavailability ( $F\%$ ) to 50% and a short elimination half-life of 4 h; parameters necessitating its frequent administration. The current study was therefore, designed

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to formulate and optimize the transfersomal TiM gel for transdermal delivery. TiM loaded transfersomal gel was optimized using two  $2^3$  full factorial designs; where the effects of egg phosphatidyl choline (PC): surfactant (SAA) molar ratio, solvent volumetric ratio, and the drug amount were evaluated. The formulation variables; including particle size, drug entrapment efficiency (%EE), and release rate were characterized. The optimized transfersomal gel was prepared with 4.65:1 PC:SAA molar ratio, 3:1 solvent volumetric ratio, and 13 mg drug amount with particle size of 2.722  $\mu\text{m}$ , %EE of 39.96%, and a release rate of 134.49  $\mu\text{g}/\text{cm}^2/\text{h}$ . The permeation rate of the optimized formulation through the rat skin was excellent (151.53  $\mu\text{g}/\text{cm}^2/\text{h}$ ) and showed four times increase in relative bioavailability with prolonged plasma profile up to 72 h compared with oral aqueous solution. In conclusion, a potential transfersomal transdermal system was successfully developed and the factorial design was found to be a smart tool, when optimized.

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## Introduction

Timolol maleate is a  $\beta$ -adrenergic blocking agent that exhibits an anti-hypertensive activity, protects against angina pectoris, and myocardial infarction. Due to its short elimination half-life (4 h), it is orally administered twice daily. Additionally; because of poor bioavailability (50%), a high oral dose of 10–60 mg/day was required. As an adverse effect, bronchospasm was reported in some patients [1].

Transdermal delivery represents an attractive solution to oral problems. It bypasses the liver first pass effect; hence the bioavailability is expected to be increased. Additionally, it can be simply terminated and removed from the skin, if any of the side effects show up. Furthermore, the use of the vesicular system in the transdermal drug delivery may sustain the release of the drug, thus lowers its frequency of administration [2]. Despite the many advantages of the skin as a site of drug delivery, only few drugs are currently available in the market as transdermal delivery systems. This is because the inherent limitation of transdermal drug absorption, which is imposed by the outermost layer of the skin, the stratum corneum (SC) [3]. From 1991, several researches were focused on transfersomes in transdermal drug delivery system to overcome this intrinsic barrier. Transfersomes can penetrate efficiently various transport barriers, even through the pores or constrictions that would be confining for other particulates of comparable size. This capability is due to the self-adaptable and extremely high deformability of the transfersomes' membrane [4]. In contrast to other methods permeating the skin; transfersomes create drug depots in the skin that can slowly and gradually deliver the material under the skin and/or the systemic circulation without invasion [5]. Transfersomes are complex aggregate, composed of phospholipids, surfactant, and water; prepared by thin film hydration or modified hand shaking, lipid film hydration technique [5].

Analysis and understanding the appropriate combination of independent process and/or formulation variables (factors), which produce the optimized product can be established by statistical design of experiment tools, such as factorial designs. It is considered as the most effective way in estimating the influence of individual process variables with minimum experimentation and time, where all factors are tested in all possible combinations [6]. The aim of the present study was therefore, to develop timolol maleate transfersomal gel formulation by

thin film hydration method for transdermal uses. Two  $2^3$  full factorial designs were employed to optimize and explore the effect of three formulation variables; including phosphatidylcholine: surfactant molar ratio, the solvent volumetric ratio, and the drug amount using two different surfactants (Tween 80 and Span 80). The aforementioned effects were evaluated on each of the particle size of the vesicles, the percentage entrapment efficiency of the drug, and the release rate through synthetic membrane. The optimized formulation was subjected to permeation studies using shaved rat skin and in vivo pharmacokinetics studies were carried out on Wistar rats on the optimized formulation; comparing the results with the oral solution.

## Material and methods

### Materials

Timolol maleate (TiM) was a gift from Sedico Company (Giza, Egypt). L- $\alpha$ -phosphatidylcholine (PC) (type IV-S) and Span 80 (S80) were purchased from Sigma Aldrich (St. Louis, MO, USA). Tween 80 (T80) was obtained from Scharlau Chemie (Sentmenat, Spain). Carbopol<sup>®</sup> 934 was supplied by Lubrizol Corporation (Ohio, USA). Naproxen sodium powder was a generous offer from El-Nile Pharmaceutical Chemical Company (Cairo, Egypt). All other chemicals and solvents were of pharmaceutical grade.

### Preparation of transfersomes

Transfersomes were prepared by dry thin film hydration method [7]. A mixture of PC and surfactant (SAA) with different ratios was dissolved in 12 mL mixture of chloroform and methanol to form 5% w/v solution. The solvent was removed by rotary evaporation at 55 °C under reduced pressure (Heidolph 2, Schwabach, Germany) till a thin film is produced. The film was hydrated with 10 mL of phosphate buffer saline (PBS) pH 7.4, containing the drug. The formed suspension was subsequently sonicated for 10 min using bath type sonicator at 900H at temperature 25 °C (Jiotech UC-10, Serangoon, Singapore). The suspension was left overnight for maturation of vesicles and kept under vacuum to ensure the removal of residual solvent.

### Experimental design

Two  $2^3$  full factorial designs were employed using Design-Expert 7.0.0 software (Stat-Ease Inc., USA), one using T80 and the other using S80 as the SAA. In these designs, three independent formulation variables were studied to evaluate their individual and combined effects; PC:SAA molar ratio ( $X_A$ ), chloroform: methanol volumetric ratio ( $X_B$ ), and amount of drug added ( $X_C$ ), each at two levels. The experimental trials were performed at all eight possible combinations with 3 times replication for each transfersomal system. The effect of particle size (P.S.), percentage entrapment efficiency (%EE), and the timolol maleate (TiM) release rate through synthetic membrane on transfersomes performance and characteristics were tested and optimized. The levels of the independent variables were chosen based on the preliminary experiments (results not shown). The full factorial designs including investigated independent and dependent variables are shown in Table 1. The one-way analysis of variance (ANOVA) was applied to estimate the significance of the model ( $P < 0.05$ ) and individual response parameters.

### Morphology and vesicle size measurement

The mean vesicle size and morphology of the prepared transfersomes were determined using the optical microscope (Leica Imaging Systems, Cambridge, UK) with a digital camera (JVC, Victor Co, Yokohama, Japan) [8]. A thin layer of transfersomal formulation was spread on a slide and examined after placing the cover slip. The average size of at least 100 particles was measured.

### Determination of entrapment efficiency of TiM in transfersomes

One mL of the previously prepared suspension was centrifuged at 18,000g for 1 h at a temperature of 4 °C using cooling centrifuge (Megafuge® 16R, Hanau, Germany), followed by washing the precipitate twice with PBS at pH 7.4 [9–11]. The free TiM concentration ( $C_f$ ) in the resulting supernatant and the resulting washing solution was assayed spectrophotometrically at 294 nm after filtration and suitable dilution. The %EE of the drug was calculated from the following equation as follows:

$$\%EE = [(C_t - C_f)/C_t] \times 100$$

where  $C_t$  is the total added theoretical concentration of TiM used in the preparation of transfersomes and  $C_f$  is the concentration of untrapped TiM [12].

### Preparation of transfersomal gel

Transfersomal gel was prepared by adding 0.5 g Carbopol® portion wise by sprinkling to 4 mL of the previously prepared suspension and stirring 8-wise direction until a gel was formed.

### In vitro release study

*In vitro* release studies were carried out using vertical diffusion Franz cells (Hanson Research Corp, CA, USA) with an effective diffusion area of 1 cm<sup>2</sup>. The receptor's compartment volume was 7 mL (PBS, pH7.4), maintained at 37 °C ± 0.5 and stirred by a magnetic bar at 500 g. The donor compartment was separated from the receptor compartment by cellophane membrane (cut-off 12,000–14,000) (Spectrum Medical Inc., Los Angeles, CA, USA). Sample (1 g) of the gel was placed in the donor compartment. Four hundred µL aliquots were withdrawn from the sampling port at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18 and 24 h and substituted with fresh buffer, to maintain a constant volume and then analyzed spectrophotometrically at 294 nm. The calculated TiM concentration was plotted as a function of time. The rate of drug release was calculated from the slope of the initial portion of the graph [12–14].

### In vitro rat skin permeation study

*In vitro* permeation study was conducted on the optimized formulations from the two factorial designs using vertical type diffusion cell as described above. However, instead of synthetic cellophane membrane a shaved rat skin was used [13]. The permeation study was applied under non-occlusive conditions. Samples were collected at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 12, 18, and 24 h of time interval, for the two optimized formulations. The calculated TiM concentration was plotted as a function of time (h) for each formulation.

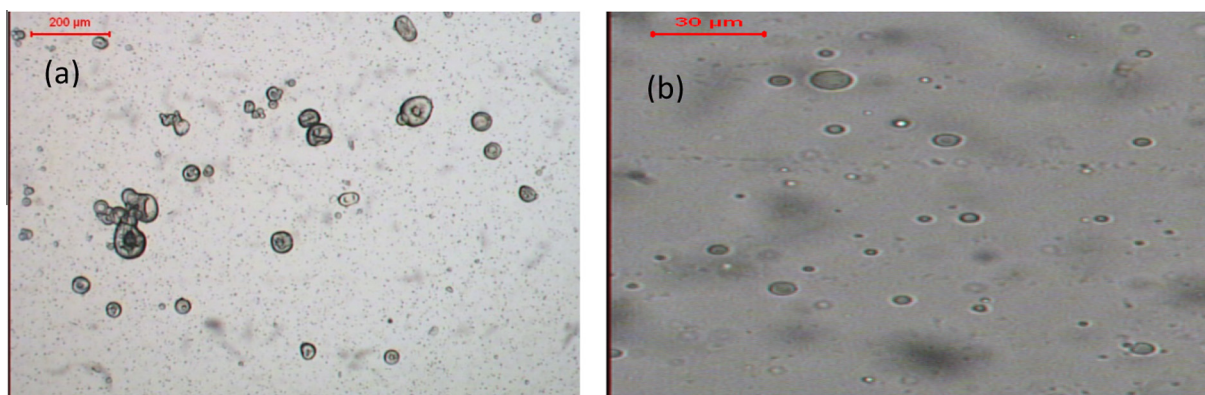
### In vivo pharmacokinetics study

#### Study protocol

*In vivo* studies were performed on male Wistar Albino rats to compare the absorption of transdermal gel with oral absorption of TiM aqueous solution. Rats were housed in animal facilities under standard laboratory conditions prior to experimentation. All investigations were performed after approval by the MSA University ethical committee (Ethical committee approval No. AE/22/H8). Twenty rats (weighing from 200 to 250 g) were used and divided into two groups; 10 rats each. The hair on the abdominal side of one group was cut off using scissors and the gel was spread over the back non-occlusively. The other group was given TiM aqueous solution orally using a plastic syringe. Gel and oral solution equivalent to 5 mg TiM were given to both groups. The skin was measured for the presence erythema and edema according to the following scaling system; the erythema scale was as follows: 0, none; 1, slight; 2, well defined; 3, moderate, and 4, scar formation, whereas the edema scale was: 0, none; 1, slight; 2, well defined; 3,

**Table 1** Variables in factorial design.

	Levels used	
	-1	1
<i>Independent variables</i>		
$X_A$ : PC:SAA molar ratio	3:1	9:1
$X_B$ : CHCl <sub>3</sub> :CH <sub>3</sub> OH volumetric ratio	1:1	3:1
$X_C$ : Drug amount	7 mg	13 mg
<i>Dependant variables</i>		
$Y_1$ : Particle size (µm)		
$Y_2$ : Percentage entrapment efficiency		
$Y_3$ : Release rate (µg/cm <sup>2</sup> /h)		



**Fig. 1** Light photos for the optimized transfersomal formulae (a) using T80 and (b) using S80 as the surfactant.

moderate, and 4, severe. Composite of erythema and edema scores was rated as follows: 0, none; 1–2 mild; 3–5, moderate; and 6–8, severe irritation [15].

To measure the concentrations of TiM, blood samples (500 µL) were obtained from the retinovascular plexus of the eye using heparinized capillaries. Samples were centrifuged at 4000g for 10 min to obtain plasma, which stored at  $-20^{\circ}\text{C}$  in labeled tubes pending HPLC analysis.

#### HPLC assay

The quantitative determination of drug was performed by a validated HPLC method [16], using acetonitrile: 0.2% triethylamine (60:40, v/v) (pH 2.75 adjusted with 85% phosphoric acid) as a mobile phase delivered at 1.0 mL/min. The HPLC system equipped with degasser (G1379A), quaternary pump (G1311A), auto-sampler with 50 µL injection loop, column thermostat (G1316A) and UV detector (G1315C). The column oven temperature was kept at  $45^{\circ}\text{C}$  and the peak response was monitored at a wavelength of 294 nm. The mobile phase system control and data acquisition were made with the Agilent Chem Station Version A 10.02 (Agilent Technologies, Munich, Germany). Standard addition technique was adopted so as to detect the small quantities of the drug in the samples [16,17].

#### Pharmacokinetics and statistical analysis

Pharmacokinetics parameters were calculated using the plasma concentration vs. time, using non-compartmental analysis (Kinetica<sup>®</sup> 5, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

#### Results and discussion

##### Preparation of transfersomes

Based on preliminary experiments, transfersomes were prepared by thin film hydration method rather than reverse phase evaporation (REV), because it produces multilamellar vesicles (MLV) with higher drug loading [9].

##### Morphology

Fig. 1 represents the photomicrographs of the optimized formulations using T80 and S80. It shows the outline and core of the well-identified spherical shaped vesicles, displaying the

**Table 2**  $2^3$  full factorial design layout.

Formula code	Independent variable levels in coded form			SAA	Dependant variables		
	$X_A$	$X_B$	$X_C$		$Y_1 \pm \text{S.D.} (\mu\text{m})$	$Y_2 \pm \text{S.D.}$	$Y_3 (\mu\text{g}/\text{cm}^2/\text{h})$
1T	1	-1	-1	T80	$1.61 \pm 0.042$	$49.9 \pm 0.459$	197.33
2T	-1	1	-1		$1.73 \pm 0.042$	$11.6 \pm 0.325$	130.29
3T	-1	-1	1		$2.74 \pm 0.084$	$69.2 \pm 1.209$	186.89
4T	1	1	-1		$1.45 \pm 0.353$	$32.73 \pm 0.855$	168.13
5T	1	-1	1		$1.5 \pm 0.141$	$37.68 \pm 0.318$	166.42
6T	-1	1	1		$2.42 \pm 0.042$	$33.85 \pm 0.77$	113.38
7T	1	1	1		$3.645 \pm 0.403$	$7512 \pm 2.715$	46.26
8T	-1	-1	-1		$3.205 \pm 0.106$	$85.2 \pm 0.728$	125.4
1S	1	-1	-1	S80	$1.925 \pm 0.1$	$47.5 \pm 0.721$	46.274
2S	-1	1	-1		$3.85 \pm 0.07$	$11.67 \pm 1.918$	33.257
3S	-1	-1	1		$3.5 \pm 0.14$	$60.559 \pm 0.607$	30.673
4S	1	1	-1		$2.985 \pm 0.035$	$29.7 \pm 1.007$	60.28
5S	1	-1	1		$3.36 \pm 0.127$	$25.94 \pm 1.018$	82.458
6S	-1	1	1		$3.2 \pm 0.48$	$26.98 \pm 0.084$	176.32
7S	1	1	1		$1.72 \pm 0.113$	$67.2 \pm 1.343$	31.556
8S	-1	-1	-1		$2.655 \pm 0.289$	$50.1 \pm 2.186$	69.682



retention of sealed vesicular structures, which are nearly homogenous in shape.

*Experimental design*

Based on the factorial design of experiment, the optimization technique encompassed the generation of model equations for the investigated dependent variables over the experimental design, to determine the optimum formulation(s). Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries. A positive sign in front of the terms indicates synergistic effect, while negative sign indicates antagonistic effect of the factors. The results of dependent variables were represented in Table 2. Table 3 shows the one-way ANOVA, which estimates the significance of the model ( $P < 0.05$ ) and individual response parameters [18].

*Vesicle size*

The mean vesicle size using T80 was ranged from 1.45 to 3.64  $\mu\text{m}$ , whereas that of S80 ranged between 1.72 and 3.85  $\mu\text{m}$  (Table 2). Eqs. (1) and (2) represent the linear regression models for particle size (P.S.) using T80 and S80 transfersomes, respectively, as obtained from factorial design study.

$$\begin{aligned} \text{Particle size using T80} &= 2.29 - 0.24X_A + 0.024X_B \\ &+ 0.29X_C + 0.47X_{AB} \\ &+ 0.23X_{AC} + 0.43X_{BC} \end{aligned} \tag{1}$$

[where  $F = 21.9$ ,  $P < 0.0001$  and  $R^2 = 0.93$ ]

$$\begin{aligned} \text{Particle size using S80} &= 2.9 - 0.4X_A + 0.039X_B \\ &+ 0.46X_C - 0.18X_{AB} - 3.12X_{AC} \\ &- 0.52X_{BC} \end{aligned} \tag{2}$$

[where  $F = 15.28$ ,  $P < 0.0001$  and  $R^2 = 0.91$ ]

The P.S was reduced by increasing the PC: SAA molar ratio as can be deduced from the negative coefficient of  $X_A$ . This might be attributed to the decreases in the SAA, which lead to incomplete maturation of vesicles and thus reduction in their sizes [8]. On the other hand, with increase in the drug amount, the particle size was increased, which may be due to the increases in drug loading [19]. As can be deduced, using different surfactants did not have a significant difference on the particle size being prepared by the same method. This finding was in accordance with that reported by Song and Kim [11].

*Entrapment efficiency*

Entrapment efficiency is the percent of the total drug incorporated into the transfersomes [13]. Eqs. (3) and (4) represent the linear regression models for %EE using T80 and S80 transfersomes, respectively, as obtained from factorial design study.

**Table 3** Sum of squares, degree of freedom, mean squares,  $F$ -values and  $P$ -values for the Model Coefficients Estimated from the Factorial Study for the measured dependent variables using T80 and S80.

Term		Sum of squares	d.f.	Mean squares	$F$ value	$P$ value	Sum of squares	d.f.	Mean squares	$F$ value	$P$ value
<i>Particle size</i>											
$X_A$	T80	0.89	1	0.89	12.14	0.0069	S80	2.58	1	2.58	0.0003
$X_B$		9.025	1	9.025	0.12	0.7342		0.025	1	0.025	0.5973
$X_C$		1.33	1	1.33	18.14	0.0021		0.033	1	0.033	0.5416
$X_{AB}$		3.57	1	3.57	48.57	<0.0001		0.54	1	0.54	0.0305
$X_{AC}$		0.86	1	0.86	11.76	0.0075		1.562E-004	1	1.562E-004	1.888E-003
$X_{BC}$		2.99	1	2.99	40.69	0.0001		4.40	1	4.40	53.17
$X_{ABC}$		0.33	1	0.33	7.98	0.0523		0.36	1	0.36	7.61
<i>%EE</i>											
$X_A$	T80	4.01	1	4.01	0.74	0.4105	S80	123.52	1	123.52	1.54
$X_B$		1899.09	1	1899.09	353.11	<0.0001		576.95	1	576.95	7.18
$X_C$		269.67	1	269.67	50.14	<0.0001		479.42	1	479.42	5.97
$X_{AB}$		4184.60	1	4184.60	778.08	<0.0001		2334.17	1	2334.17	29.05
$X_{AC}$		209.05	1	209.05	38.87	0.0002		9.47	1	9.47	0.12
$X_{BC}$		2151.24	1	2151.24	400.00	<0.0001		1012.91	1	1012.1	12.61
$X_{ABC}$		31.57	1	31.57	15.00	0.547		700.78	1	700.78	251.19
<i>Release rate</i>											
$X_A$	T80	43.86	1	43.86	2.04	0.1867	S80	1809.95	1	1809.9	294.20
$X_B$		10903.01	1	10903.01	507.98	<0.0001		1276.60	1	1276.6	207.51
$X_C$		2740.26	1	2740.26	127.67	<0.0001		3185.53	1	3185.5	517.80
$X_{AB}$		1864.30	1	1864.30	86.86	<0.0001		4907.28	1	4907.2	797.67
$X_{AC}$		11926.28	1	11926.28	555.66	<0.0001		2417.49	1	2417.4	392.96
$X_{BC}$		7277.37	1	7277.37	339.06	<0.0001		3017.20	1	3017.2	490.44
$X_{ABC}$		45.39	1	45.39	2.46	0.1556		14265.08	1	14265.08	2318.76

$$\begin{aligned} \%EE \text{ for T80} = & 49.3 - 0.5X_A - 10.89X_B + 4.11X_C \\ & + 16.17X_{AB} + 3.61X_{AC} + 11.6X_{BC} \end{aligned} \quad (3)$$

[where  $F = 270.16$ ,  $P < 0.0001$  and  $R^2 = 0.9945$ ]

$$\begin{aligned} \%EE \text{ for S80} = & 38.93 + 2.7X_A - 6X_B + 5.476X_C \\ & + 12.08X_{AB} - 0.77X_{AC} + 7.96X_{BC} \end{aligned} \quad (4)$$

[where  $F = 9.41$ ,  $P < 0.0001$  and  $R^2 = 0.86$ ]

The negative coefficient of  $X_B$  reveals the increases in the chloroform: methanol volumetric ratio, which leads to a decrease in the %EE. While an increase in the %EE was observed with the increase in the drug amount could be attributed to drug enforcement to encapsulated vesicles [12]. As can be observed, from the positive coefficient of the interaction between any of  $X_{AB}$ ,  $X_{AC}$  and  $X_{BC}$ , the %EE is synergistically increased by any interaction.

Formulations using T80 had higher %EE than S80 as shown from the coefficients of Eqs. (3) and (4), which could be attributed to the higher phase transition temperature possessed by T80 [10] that yield a higher %EE [8].

#### *In vitro* release

Fig. 2 represents the *in vitro* release profile of the drug from the formulations which followed a biphasic release. This could be attributed to the presence of free drug together with the entrapped one, which is due to the limited capacity of the lipid to accommodate large amounts of the drug leading to disposition of the free drug at the surface. This in turn lead to an initial rapid release (due to the presence of the free drug and the drug adsorbed on the surface), followed by slower sustained release phase due to diffusion of the entrapped drug through the lipid bilayers of the vesicles, which is very effective in sustaining and controlling the release of TiM. These findings were in accordance with El Zaafarany et al. [9].

Eqs. (5) and (6) represent the linear regression models for the release rate through synthetic membrane using T80 and S80 transfersomes respectively, as obtained from factorial design study.

$$\begin{aligned} \text{Release rate using T80} = & 140.93 + 1.66X_A - 26.1X_B \\ & - 13.09X_C - 10.79X_{AB} \\ & - 27.3X_{AC} - 21.33X_{BC} \end{aligned} \quad (5)$$

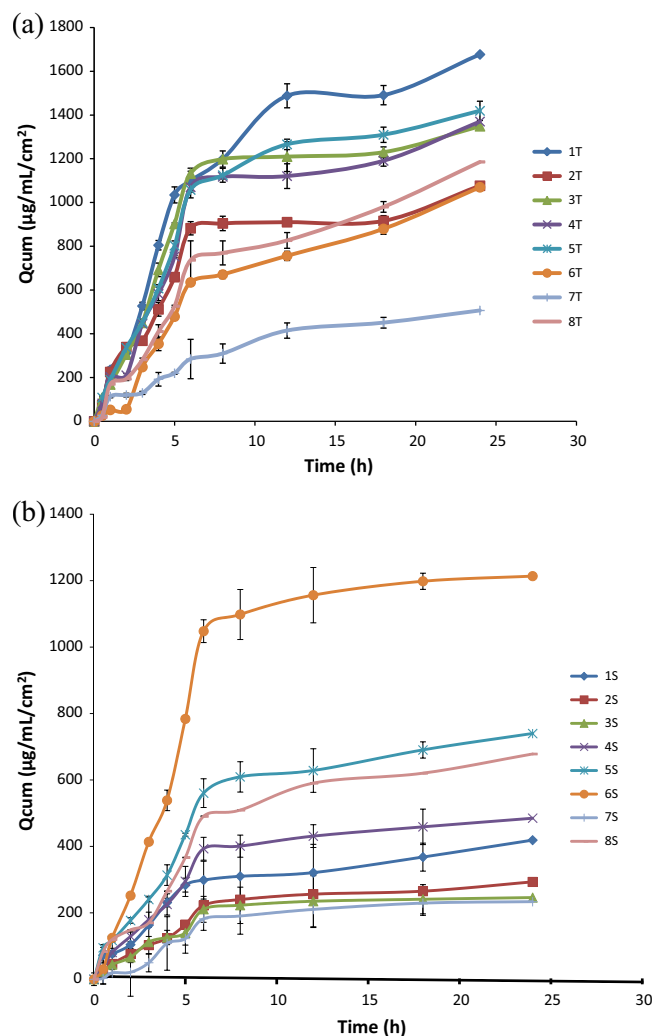
[where  $F = 269.88$ ,  $P < 0.0001$ ,  $R^2 = .9984$ ]

$$\begin{aligned} \text{Release rate using S80} = & 65.79 - 10.64X_A + 8.93X_B \\ & + 14.11X_C - 17.51X_{AB} \\ & - 12.29X_{AC} + 13.73X_{BC} \end{aligned} \quad (6)$$

[where  $F = 269.88$ ,  $P < 0.0001$ ,  $R^2 = 0.9984$ ]

It is well reported that any factor that increases the formation of the transfersomes or increases its %EE, affects the release rate, as the drug passes through the bilayers of the vesicular structure leading to a decrease in the release rate [20].

When S80 was used as the SAA, the release rate was found to be delayed by increasing the PC:SAA molar ratio. This may



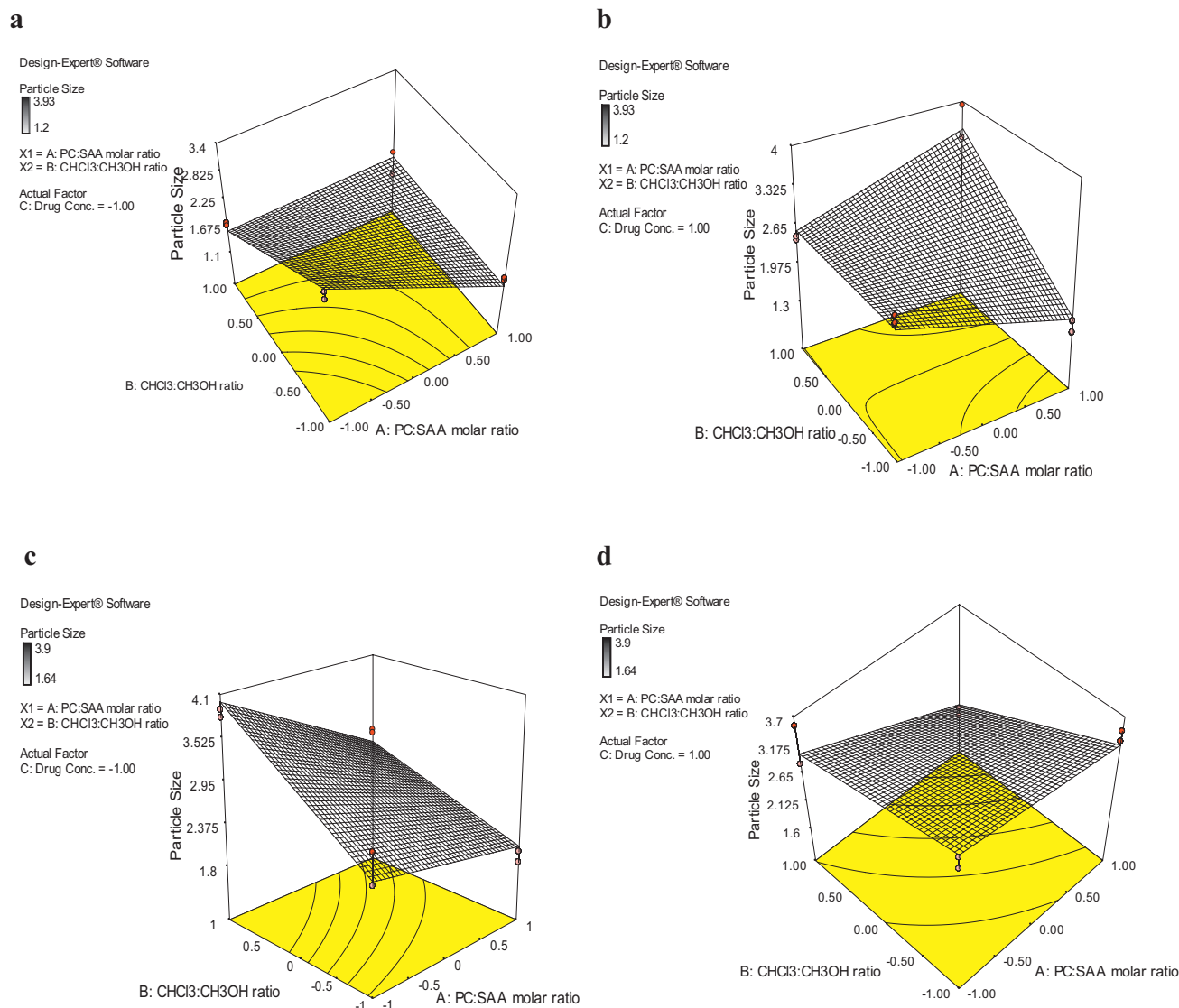
**Fig. 2** (a) *In vitro* release profiles of TiM from transfersomes from formulae (1T–8T) using T80. (b) *In vitro* release profiles of TiM from transfersomes from formulae (1S–8S) using S80.

be due to the increase in the transfersomes yield due to the increase in the body of transfersomes by increasing the PC amount, which hinder the release of the entrapped drug as well as the free one [21].

The release rate was augmented as the solvent volumetric ratio increased while using T80 and was hindered while using S80. The difference in the behavior could be attributed to the variation in hydrophilic/lipophilic balance between T80 and S80 that lead to dissimilarity in their solubility which finally lead to the difference in the release rate.

Also the release rate was found to be reduced by increasing the amount of the drug added when T80 was used which may be due to the increase in the %EE, while when S80 was used; the release rate was found to be accelerated.

It is worth to note that all studied independent variable (factors) and their binary interactions were significant for all studied dependent variables (responses) with some exceptions; where when T80 was the used SAA, the effect of  $X_A$  was insignificant on both the %EE and the release rate and the effect of  $X_B$  was insignificant on the particle size.



**Fig. 3a** 3D of particle size, (a) and (b) using T80, (c) and (d) for using S80 (a) and (c) for low level of  $X_C$  and (b) & (d) for high level of  $X_C$ .

While using S80; the interaction of  $X_{AC}$  was insignificant on the particle size and the %EE, and the effect of  $X_A$  was insignificant on the %EE and finally the  $X_B$  and  $X_C$  had insignificant effects on the particle size. This may reflect the good choice for the studied variables.

### 3D surface plots

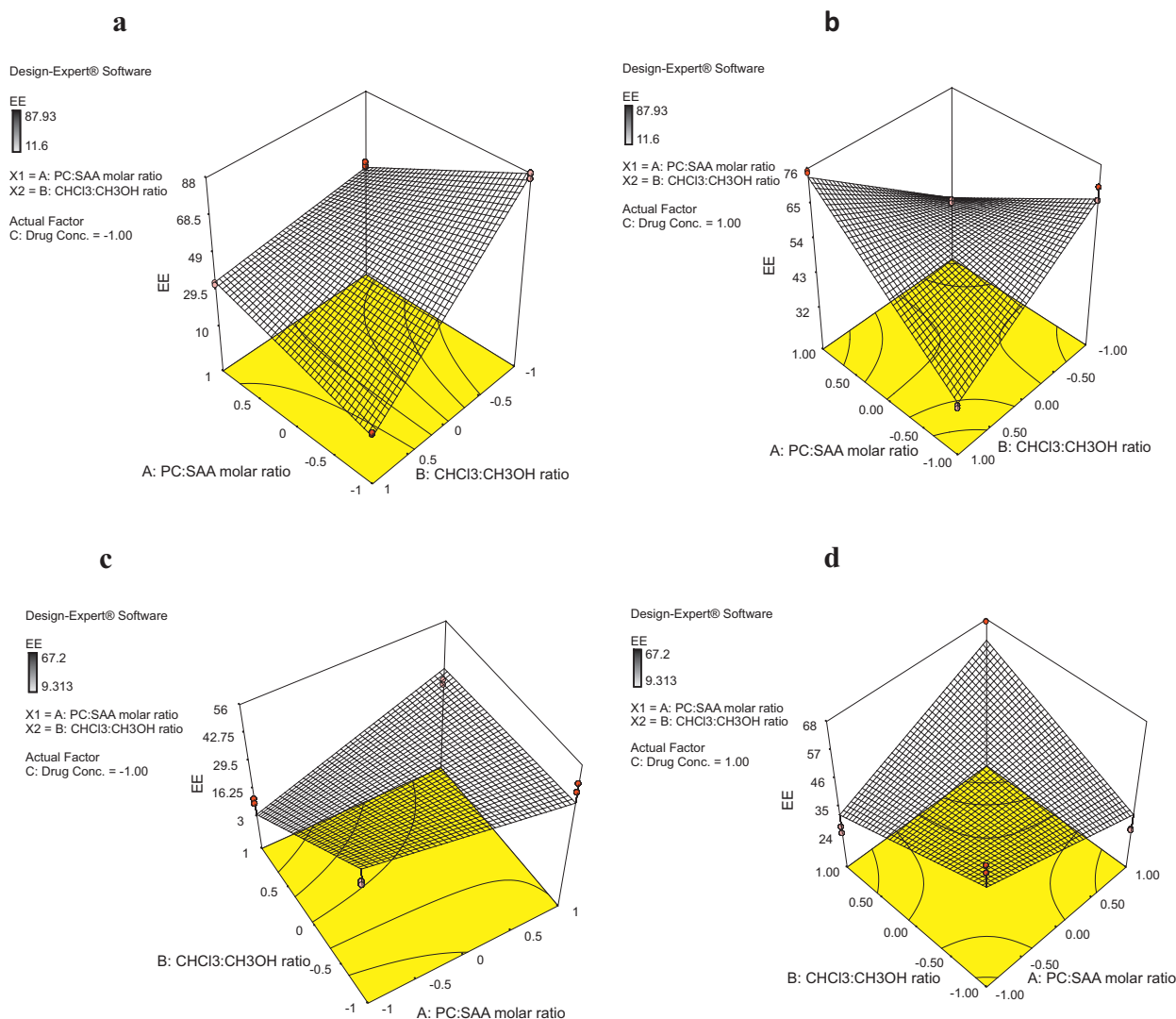
3-D surface plots were obtained by fixing the  $X_C$  factor at its high and low level and varying ( $X_A$ ) and ( $X_B$ ) over the range used in the factorial study. Fig. 3 depicts 3-D plots which show the effects of  $X_A$  and  $X_B$  on P.S., %EE and the release rate.

### Optimization

A numerical optimization technique using the desirability approach was employed to develop new optimized formula-

tions with desired response. The optimal values of responses were obtained by numerical analysis using the Design-Expert 7.0.0. software. Our constraints were the smallest particle size, highest %EE and the slowest release rate. The optimized formulation using T80 contained 8.7:1 PC:SAA molar ratio, 1:1 solvent volumetric ratio and 7 mg drug amount, with desirability 0.8, while using S80 4.65:1 PC:SAA molar ratio, 3:1 solvent volumetric ratio and 13 mg drug amount, with desirability 0.595.

A new batch was prepared and evaluated from the software at determined levels. Results depicted in Fig. 4 show the release rate of the optimized formulations. The results of expected and observed values for the optimized transfersomal formulations are shown in Table 4. The observed results of independent variables were very close to the expected values as shown from the small residual values. This proves the validation of our models.



**Fig. 3b** 3D of %EE, (a) and (b) using T80, (c) and (d) for using S80 (a) and (c) for low level of  $X_C$  and (b) & (d) for high level of  $X_C$ .

### *In vitro permeation*

The conditions were conducted non-occlusively as recommended by other [22]. This may be due to the presence of the non-chemical gradient, the hydration gradient, which is created by the difference in the total water content between the skin surface and the interior of the skin. When the transdermal formulation was placed on the skin, it is partly dehydrated by the water evaporation loss which creates the hydration gradient, which in turn enforces the transdermal formulations to enter to the skin to avoid complete hydration. This gradient can only be achieved when the formulation is applied under non-occlusive conditions to allow the water loss from the surface of the skin to create the driving force for permeation. This movement can be achieved easily due to the high flexibility of the transdermal formulations [22]. Another mechanism is that the transdermal formulations can act as a penetration enhancer, as suggested by El Zaafarany et al. [9]. This action is achieved as the vesicles bilayers enter the SC and modify the intercellular lipids, which

raise its fluidity and weakness and thus can overcome the barrier property of the skin. So these findings indicate that both the entrapped and the free drugs are carried through the SC and deep to different layers of the skin.

The permeation rate was less than the release rate, which gives an indication of the depot action of the viable skin layers, allowing the release to be more sustainable [14].

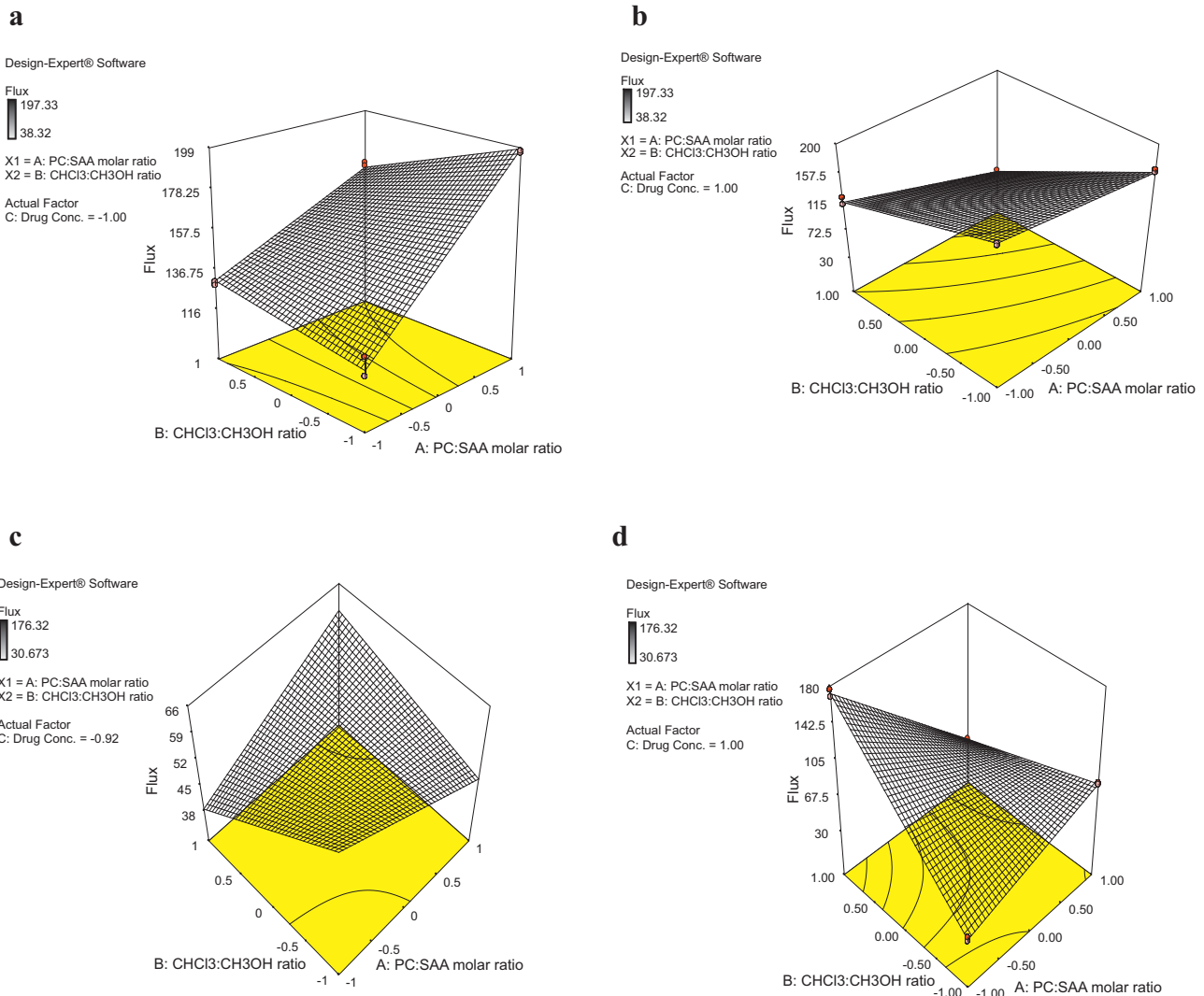
It could be inferred from Fig. 5 that S80 had better permeation than T80. This finding was in line with Jain et al. [14], who attributed the reason to the higher affinity with the lipid bilayer.

### *In-vivo pharmacokinetics study*

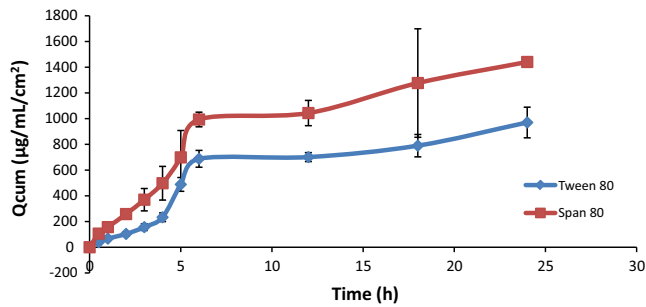
#### *Skin irritation study*

After the removal of the dosage form, no signs of erythema or edema (all had 0 scales) were observed at the application sites. This means that the preparations were well tolerated by the skin throughout the whole tested period.





**Fig. 3c** 3D of release rate, (a) and (b) using T80, (c) and (d) for using S80 (a) and (c) for low level of  $X_C$  and (b) & (d) for high level of  $X_C$ .



**Fig. 4** *In vitro* release profile of TiM from optimized formulations.

**Table 4** The observed and the predicted values for the optimized transfersomal formulations.

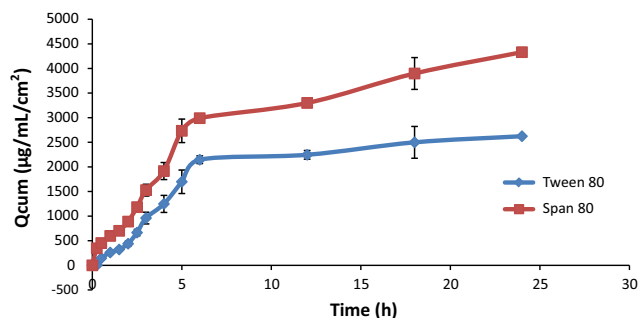
Dependent variable	Expected	Observed	Residual <sup>a</sup>
<i>(a) Using T80</i>			
P.S. (µm)	1.46	1.26	0.2
%EE	48.0175	43.678	4.3395
Release rate (µg/cm <sup>2</sup> /h)	198.55	166.51	32.04
<i>(b) Using S80</i>			
P.S.(µm)	2.722	2.4	0.322
%EE	39.96	33.19	6.77
Release rate (µg/cm <sup>2</sup> /h)	134.49	151.53	-17.04

<sup>a</sup> Residual = expected-observed.

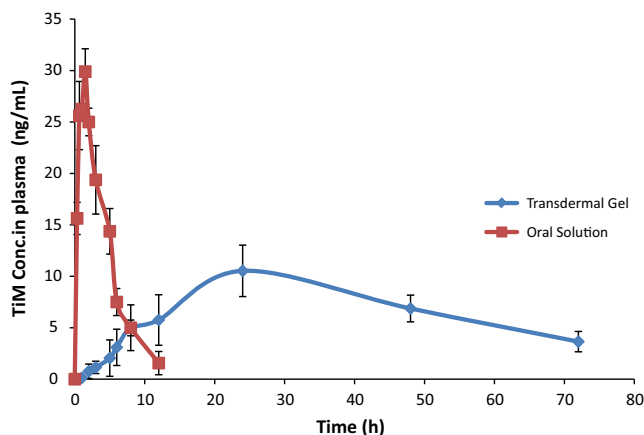
*Pharmacokinetics and statistical analysis*

Plasma profile of TiM from transfersomal gel through rat skin and absorption from single oral dose administration is shown in Fig. 6 and Table 5. After oral administration, the peak plasma concentration,  $C_{max}$ , was 29.9 ng/mL and the  $t_{max}$

was 1.5 h. With transdermal gel, prolonged plasma concentration for over 72 h was observed as indicated by decreased  $C_{max}$  (10.53 ng/mL) and delayed  $T_{max}$  (24 h). The mean  $AUC_{0-24}$  and  $AUC_{0-\infty}$  of the optimized TiM gel were increased 4.45 and 3.39 folds compared to TiM oral solution, respectively. This increase in the bioavailability could be due to bypassing



**Fig. 5** Permeation rate/cm<sup>2</sup> of the optimized formulations using T80 and S80.



**Fig. 6** Average plasma concentration time profile following administration of different formulae.

**Table 5** Mean pharmacokinetic parameters of TiM oral solution and optimized transersomal gel.

Pharmacokinetics parameter	TiM oral solution	TiM transersomal gel
$C_{max}^*$ (ng/ml)	29.90	10.53
$T_{max}^*$ (h)	1.50	24
$AUC_{0\text{ to }24}^*$ (ng/µL * h)	0.142251	0.633585
$AUC_{0\text{ to }∞}^*$ (ng/µL * h)	0.136219	0.462407

\* Significant at  $P < 0.1$ .

the first pass effect with transdermal preparation. The prolonged plasma profile and improved bioavailability of TiM from the optimized formulation could suggest the reduction of both frequency of administration and dose that would lead to good patient compliance, lower cost, and reduction of the dose related side effects.

## Conclusions

Transdermal transersomal formulation of TiM can be considered as a golden solution, which overcomes most of the oral problems. It sustained the release of the drug up to 72 h and increased the relative bioavailability in comparison with oral

route. Additionally, factorial design was found to be a smart tool in its optimization. Finally, transdermal transersomal timolol maleate gel could be a potential candidate for the treatment of hypertension.

## Conflict of Interest

The authors have declared no conflict of interest.

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