


# Transdermal Delivery of Telmisartan: Formulation, in vitro, ex vivo, Iontophoretic Permeation Enhancement and Comparative Pharmacokinetic Study in Rats

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**Purpose:** The purpose of this study was to prepare telmisartan transethosomes, incorporate them into a gel, evaluate them for in vitro drug release and in vivo permeation using iontophoresis to enhance their transdermal delivery.

**Materials and Methods:** TE formulae were prepared using various surfactants (SAAs), different ethanol concentrations, and different phospholipid-to-SAA ratios with different cholesterol ratios, characterized according to their entrapment efficiency percentage (EE %), zeta potential (ZP), particle size (PS), and polydispersity index (PDI). The optimum three formulae were incorporated into a gel, evaluated physically, in vitro dissolution, and ex vivo drug permeation using rat skin and Iontophoresis was performed on the best formula.

**Results:** The optimum three formulae (F29, F31, F32) had an EE% of  $97 \pm 0.26\%$ ,  $89 \pm 0.25\%$  and  $88 \pm 0.17\%$ , PS of  $244 \pm 5.88$  nm,  $337 \pm 4.6$  nm and  $382.2 \pm 3.06$  nm, PDI of  $0.57 \pm 1.9$ ,  $0.5 \pm 1.4$  and  $0.63 \pm 2.2$  and ZP of  $-31.6 \pm 1.59$  mV,  $-28.3 \pm 3.79$  mV and  $-31 \pm 5.65$ , respectively. Selecting F29 for in vivo study by iontophoretic enhancement, C<sub>max</sub> was increased by 1.85 folds compared to the commercial oral tablet and by 1.5 folds compared to transdermal gel. T<sub>max</sub> decreased by half using iontophoresis compared to commercial tablets and transdermal gel.

**Conclusion:** The transethosomal formulation of telmisartan enhanced its transdermal absorption and increased its bioavailability as well. Iontophoresis was used to increase maximum plasma concentration and reduce T<sub>max</sub> by half.

**Keywords:** telmisartan, TEL, entrapment efficiency, EE, transethosomes, iontophoresis

## Introduction

Telmisartan is a selective angiotensin-II receptor blocker, which reduces blood pressure. It lowers the vasoconstriction effect and the impact of aldosterone emissions of angiotensin-II by particularly obstructing the AT1, adrenal gland receptor signals and smooth muscle vascular system.<sup>1</sup> Telmisartan illustrates a poorly-water-solubility behavior as it is assigned as a class II drug, which hinders its permeation through lipid bilayers in humans. Maximum plasma concentrations (C<sub>max</sub>) of telmisartan are achieved in the 1st hour through oral administration of the drug. The bioavailability of orally administered telmisartan is nonlinear (20–160 mg oral dose).<sup>2</sup> Various strategies have been recommended for improving the dissolving profile of these essentially insoluble drugs by considering the following factors:

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Derivatization of the medicine, complexation by preparing nanovesicles, microencapsulation, and spray drying, incorporate surfactants and increase the surface area exposed for dissolution.<sup>3</sup> Cubosomal and proniosomal oral tablets enhance Telmisartan bioavailability, maintain a controlled release profile, and enhance Telmisartan gastrointestinal absorption.<sup>4,5</sup> Transdermal drug delivery offers advantages over the oral route of administration, including avoidance of first-pass metabolism, good patient compliance, and smaller plasma fluctuations levels for repeated dosing, despite the challenges facing transdermal drug delivery in exerting the therapeutic action of the drug molecules due to the complexity of the skin. The skin consists of many layers: epidermis, dermis, stratum corneum, and adipose tissue. Stratum corneum (SC), the uppermost layer of the epidermis of 10–15  $\mu\text{m}$  thickness, is considered the main obstacle to drug flux.<sup>6</sup> Numerous techniques have been employed to overcome that barrier to improve transdermal drug delivery. One of these techniques is the employment of lipid vesicular formulations as skin drug delivery systems to improve the permeation of the drug across the skin barrier; Lipid vesicular systems such as ethosomes<sup>7</sup> and transfersomes. An obvious effort has been made to transfersome flexible carriers to enhance entrapment, drug load, size, and surface malleability that can efficiently penetrate, partition, and permeate the skin barrier.<sup>8</sup> Ethosomal systems are non-irritant vesicles, easily prepared, composed mainly of phospholipids and ethanol, compounds commonly found in pharmaceutical preparations. These carriers are highly efficient for the delivery of molecules with various lipophilicities through the skin, *in vitro* and *in vivo* in animal and clinical studies.<sup>9</sup> These approaches use non-toxic and biodegradable phospholipids, and for this reason, they can prolong the half-life of a drug to sustain its release. Generally, transdermal patches and gels are considered passive delivery techniques while microneedles, iontophoresis, sonophoresis, and ultrasound are considered enhanced delivery techniques.<sup>10</sup> Iontophoresis is a non-invasive physical method that can provide rapid delivery of drug molecules both locally and systemically,<sup>11,12</sup> involving the application of a low electrical potential gradient through the skin to improve molecular transport. However, Iontophoresis only improves small molecular drug transdermal delivery. To improve transdermal delivery of large molecules such as protein and DNA, iontophoresis had to be combined with other transdermal enhancement methods.<sup>13</sup>

This study aims to deliver telmisartan transdermally, by combining ethosomal gel with iontophoresis to enhance the bioavailability of the drug.

## Materials

All chemicals, reagents, and solvents were of analytical grade, obtained, and purchased from authorized sources. Telmisartan (TEL) was a gift from the International Drug Agency for Pharmaceutical Industry (IDI) (Egypt), L- $\alpha$  phosphatidylcholine (PC) from lecithin of soya was purchased from Cairo Erba Reagents (Cairo, Egypt), Span 60 (S60), sodium deoxycholate (SDC), cholesterol and cellulose membrane (12,000–14,000 MW cut off) were purchased from Sigma Aldrich Chemical Co. (Cairo, Egypt), Tween 80 (T80), potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide were purchased from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt), Chloroform, methanol and ethanol with a concentration of 95% were provided by Merck (Darmstadt, Germany), Carbopol 940 (Sigma, USA), Triethanolamine 98% was obtained from Loba Chemie (Mumbai, India).

## Methods

### Preparation of Telmisartan-Loaded Transethosomes

Preliminary screening trials were conducted before selecting the optimum condition for preparing transethosomes (TEs). Carriers were prepared by using three different amounts of PC (75, 85, and 95 mg) and several surfactants (SAAs), namely, S60, T80, and SDC at various amounts (5, 15 and 25 mg)<sup>14</sup> in the presence of cholesterol at a different weight ratio to surfactant, CH: SAA (2:1, 1:1 and 0:1) using the thin-film hydration technique (TFH).<sup>15</sup> Firstly, weight PC, SAAs, cholesterol, and 40mg of telmisartan in a round-bottom flask with a long neck, using 10 mL chloroform to dissolve the mixture. The pressure was maintained constant under vacuum (350 mbar) for 30 min to eliminate organic solvent traces. The organic phase was slowly evaporated at 60°C. This temperature is concerned to be above the lipid phase transition temperature ( $T_c$ )<sup>16</sup> using a rotary evaporator at 150 rpm (Rotavapor VV 2000; Heidolph, Schwabach, Germany) to form a clear thin film. Using 10 mL distilled water containing different concentrations of ethanol (5%, 10%, and 20%) (v/v) as a hydration media at 150rpm under normal pressure, glass beads were used for 45 min to ensure that the thin film had been hydrated completely<sup>17</sup> through these steps. The carriers' dispersion was left overnight

at 4°C to obtain mature carriers which were used for further characterization.

## Characterization and Optimization of TEL-Loaded TEs

### Determination of EE%

A 1mL of the carrier's dispersion was placed in a cooling centrifuge and centrifuged for 1 hr at 15,000 rpm and 4°C using (Sigma 3–30 KS; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany), then the supernatant was taken and diluted with buffer PH 5.5 and 7.4 separately, then analyzed at  $\lambda_{\text{max}}$  298 nm<sup>18</sup> using a UV–Vis spectrophotometer (Shimadzu UV1650 Spectrophotometer; Shimadzu Corp., Kyoto, Japan); the results were the same. Each test was performed in triplicate and recorded as a mean  $\pm$ SD. The entrapment efficiency was the ratio of the amount of the drug entrapped into the vesicle to the total amount of the drug and was figured by the following equation:<sup>4,19</sup>

$$\%EE = \frac{\text{total amount of drug} - \text{amount of free drug}}{\text{total amount of drug}} \times 100$$

### Determination of Particle Size (PS), Polydispersity Index (PDI) and Zeta Potential (ZP)

The mean PS, PDI, and ZP of carrier dispersions were determined for the prepared formulae by a dynamic light scattering technique using a Malvern Zetasizer 2000 (Malvern Instruments Ltd., Malvern, UK). The measurements were performed after dilution<sup>20</sup> (10 folds) with phosphate buffer 5.5 and 7.4 separately (The results were the same at PH 7.4 and 5.5).

The ZP was evaluated by monitoring the particle electrophoretic movement in the electrical field. The electrophoretic mobility was converted into ZP using the Smoluchowski equation. All measurements were repeated three times  $\pm$ SD.

### Evaluate the Influence of Different Formulation Parameters Through Factorial Design

A factorial design was used to determine the different variables' influence on the features of telmisartan transethosomal vesicles using a minimum number of experimental runs. In the chosen design, four factors were assessed: each of 3 levels (X1: SAA type), (X2: PC: SAA ratio), (X3: cholesterol: SAA ratio), and (X4: %conc. of ethanol). The EE% (Y1), PS (Y2), PDI (Y3), and ZP (Y4) were designated as dependent variables (Table 1). The experimental trials were done using 32 combinations for preparing TEL-loaded TEs, as detailed in the [Supplementary Material](#). To analyze the test results to source

**Table 1** Factorial Design Used for the Optimization of TE Formulations

Factors (Independent Variables)	Levels
X1: SAA type	S60 T80 SDC
X2:PC:SAA ratio	95:5 85:15 75:25
X3:Additives (Cholesterol:SAA ratio)	2:1 1:1 0:1
X4: %conc. of Ethanol	5% 10% 20%
Responses (Dependent Variables)	Desirability Constraints
Y1: EE%	Maximize
Y2: PS(nm)	Minimize
Y3: ZP(mV)	Maximize (as absolute value)
Y4:PDI	Minimize

**Abbreviations:** EE%, entrapment efficiency percentage; PC, phospholipid; PDI, polydispersity index; PS, particle size; S60, span 60; SAA, surfactant; SDC, sodium deoxycholate; T80, Tween 80; TE, transethosome; ZP, zeta potential.

independently the main effects of these factors, Design-Expert software version 11 (Stat Ease, Inc., Minneapolis, MN, USA) was used, followed by ANOVA to determine the significance of each factor (Table 1).

## Optimization of Prepared Transethosomes

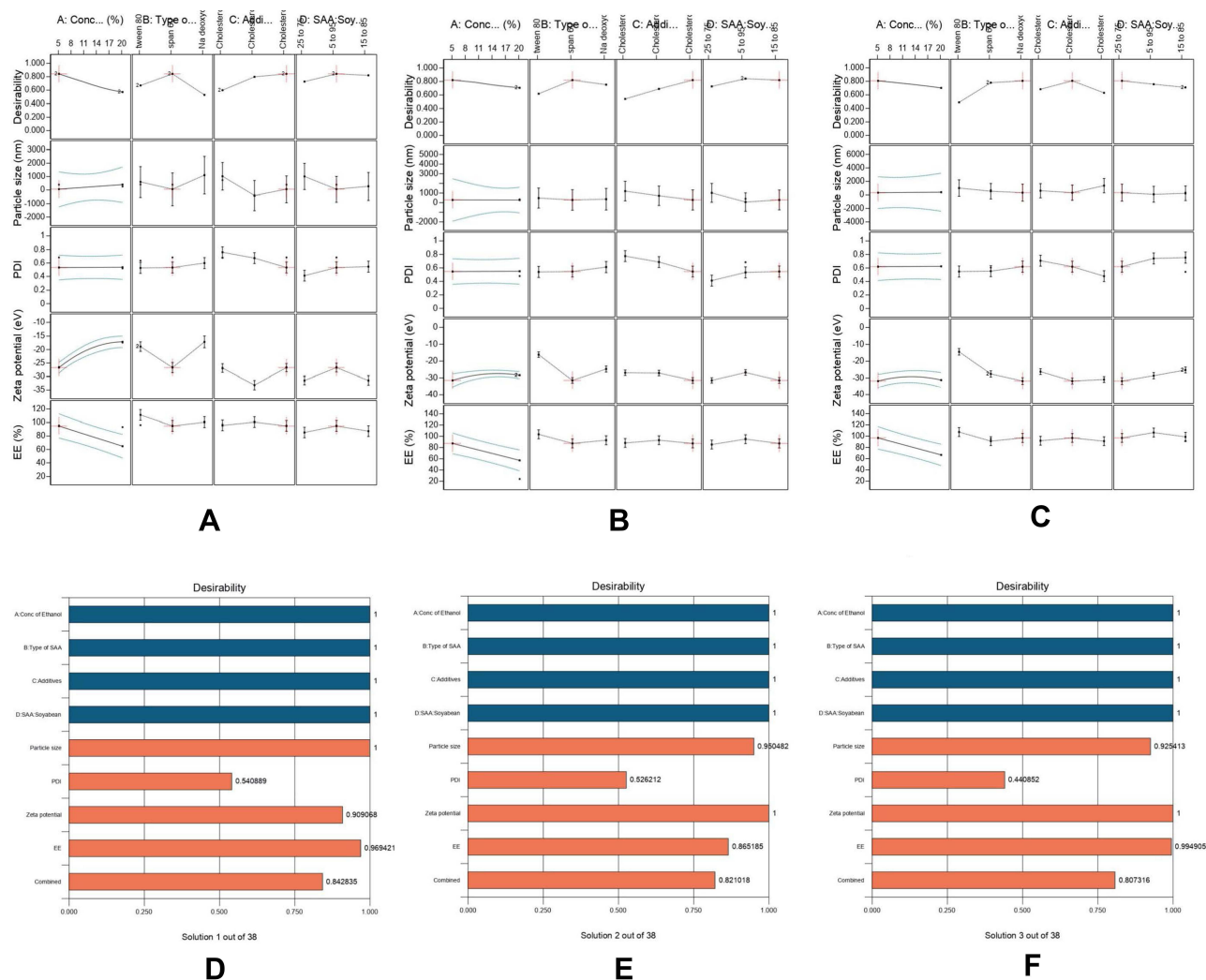
For determining the optimum formula to be selected for further investigations, select the formulae with the least PDI, PS, the highest EE%, and ZP (as an absolute value). The best formulae were chosen based on the desirability factor nearest to one (Figure 1A–F).

## Differential Scanning Calorimetry (DSC)

The thermal analysis of TEL powder, Carbopol 940, physical mixture (Carbopol 940 and Telmisartan) and formula 29 (the selected best formula) were run on differential scanning calorimetry (Shimadzu DSC-50) equipped with an intercooler 1P. A standard 20 $\mu$ L aluminum pan was used to place the sample in. Nitrogen gas had been eluted at a rate of 20 mL/min. Samples were operated at a temperature from 0 to 300°C with a gradual rise in the temperature by the rate of 10°C/min.<sup>5</sup>

## Fourier Transform Infrared (FTIR)

Using an FTIR spectrometer to detect any chemical interactions between the drug and the gel forming agent, sample of Telmisartan, Carbopol 940, and a physical mixture of Telmisartan and Carbopol 940 with a weight ratio of 1:1 were identified using (Bruker, IFS-55) in a range of 400–4000 cm<sup>-1</sup>.



**Figure 1** (A is Conc. of Ethanol, B is Type of SAA, C is Additives (Cholesterol : SAA ratio) and D is SAA : Soybean ratio), **A** Graphs of solutions for optimization of Telmisartan Ethosomes, solution 1; **B** Graphs of solutions for optimization of Telmisartan Ethosomes, solution 2; **C** Graphs of solutions for optimization of Telmisartan Ethosomes, solution 3; **D** Bar chart of desirability for optimization of telmisartan ethosomes, solution 1; **E** Bar chart of desirability for optimization of telmisartan ethosomes, solution 2; **F** Bar chart of desirability for optimization of telmisartan ethosomes, solution 3.

## Transmission Electron Microscopy (TEM)

Using a transmission electron microscope, the morphology of the optimum three formulae was studied using (JEOL JEM 1230; JEOL, Tokyo, Japan). The carrier dispersion was settled in a thin film form on a copper grid coated with carbon, stained using 2% of phosphotungstic acid,<sup>21</sup> then viewed and snapshotted.

## Preparation, Characterization and Optimization of Telmisartan Transethosomal Gel

For incorporation of the best 3 formulae into a gel, 1.5% (w/v) of carbopol 940 was soaked into the prepared

formulae (10mL of 40mg/mL) overnight before gelation, and then 0.5% (w/w) of triethanolamine was added to obtain a clear gel, adjust viscosity and PH.<sup>22</sup>

The prepared gels were characterized for appearance, viscosity, PH, in vitro drug release, and ex vivo drug permeation to select the best formula.

## In vitro Drug Release of Telmisartan

A sample of 1 gm of the prepared gel containing (40mg of telmisartan) was performed against blank formulae and telmisartan suspension into dialysis bag diffusion technique<sup>23</sup> Telmisartan was released from prepared gels using USP dissolution apparatus II (Erweka DT 600 six-axle dissolution analyzer) at a rotation speed of 50 rpm. As

a dissolution medium, 900 mL of phosphate buffer pH 7.4 was used, which was kept at 37.0°C for the entire 24-hour experiment. Ethanol (40%v/v) was added to ensure sink condition.<sup>24</sup> Test samples of 2 mL were withdrawn at predetermined time intervals of 1, 2, 4, 8, 12, and 24 h and the volume was replaced with fresh dissolution medium after each sampling, measured spectrophotometrically at  $\lambda_{\text{max}}$  298 nm. The dissolution test was done three times, and the data obtained from *in vitro* dissolution was recorded as mean  $\pm$  SD.

## Ex vivo Drug Permeation of Telmisartan

Using the same amount of drug that had been used in *in vitro* drug release tests with similar dissolution conditions, but the cellulose membrane was replaced by dorsal hairless rat skin (area 3.14 cm<sup>2</sup>); SC was facing the donor compartment.<sup>25</sup> Test samples of 2 mL were withdrawn at predetermined time intervals of 1, 2, 4, 8, 12, and 24 h and the volume was replaced with fresh dissolution medium after each sampling, % of permeated drug measured spectrophotometrically at  $\lambda_{\text{max}}$  298 nm. Dissolution was done in triplicate and the results were obtained as average values  $\pm$  SD. Permeation of TEL-loaded ethosomal gels were compared with TEL-suspension in PBS 7.4.

## Ethical Approval

All the experimental procedures used in this study were carried out according to the experimental animal protocol approved by the ethics committee of the Faculty of Pharmacy, Cairo University, Egypt with serial number: PI (2356). Wister rats weighing (200–350 g) were used to study drug bioavailability. Rats were kept under a constant temperature of 25°C $\pm$ 2°C and a relative humidity of 55% at the animal house, Faculty of Pharmacy, Cairo University, Egypt.

## In vivo Studies

Due to the similarity between human and rat skin in lipid content and water uptake features, the hairless rat skin was used to simulate transdermal permeation and topical application. The study was done using 12 Wister rats split into four groups. Group (I) was the control group, with  $n = 3$ , Group (II) received a proportionate oral dosage of 40 mg TEL, with  $n = 3$ , Group (III) received the selected TEL-loaded ethosomal gel (F29), with  $n = 3$ , and Group (IV) received the selected TEL-loaded ethosomal gel (F29) with Iontophoresis system (Chattanooga Group, a division of Encore Medical USA), with  $n = 3$ .

Anodal iontophoresis was applied. The selected gel formula was applied using the Phoresor Unit II iontophoresis device with TransQe iontophoresis electrodes of an active area of 13.4 cm<sup>2</sup> for 30 minutes (IOMED, Inc., Salt Lake City, UT, USA), the current density was adjusted to be 0.2mA/cm<sup>2</sup>,<sup>22,26,27</sup> A 0.5 mL blood sample was collected from the retro-orbital vein and put into heparinized tubes at time intervals of 0, 0.5, 1, 2, 4, 8, 12, and 24 h. The blood samples were centrifuged at 4000 rpm for 20 min for plasma separation. The plasma samples were collected and stored at -20 °C for HPLC measurement. One milliliter of acetonitrile was used to extract 0.2 mL of plasma, which was then centrifuged at 4000 rpm for 20 minutes. 100  $\mu$ L of the supernatant was diluted with 500  $\mu$ L of acetonitrile: water: acetic acid (15:85:0.1). 20  $\mu$ L was injected into the HPLC system (Hitachi LaChrome Elite, Tokyo, Japan). An isocratic mixture of methanol and acetonitrile (70:30%v/v) was selected as the mobile phase, using an Inertsil ODS column, (250, 4.6) mm, 5 $\mu$ m as a stationary phase. The mobile phase was eluted at a rate of 0.7 mL/min for 10 min.<sup>28</sup> Pharmacokinetic analysis of the plasma concentration of Telmisartan was carried out using the pharmacokinetic PK solver add-in package for Microsoft Excel 2016 applying non-compartmental analysis.<sup>29</sup> The pharmacokinetic parameters determined were: maximum plasma concentration (C<sub>max</sub>), the time for maximum plasma concentration (T<sub>max</sub>), the area under the plasma concentration versus time curve from zero to 24 hrs. (AUC<sub>0–24</sub>), the area under the plasma concentration versus time curve from zero to infinity (AUC<sub>0–∞</sub>), mean residence time (MRT), time required to achieve 50% of plasma concentration (T<sub>1/2</sub>), and terminal elimination rate constant ( $\lambda_z$ ), the area under the first moment curve from zero to twenty-four hours (AUMC<sub>0–24</sub>), and the area under the first moment curve from zero to infinity (AUMC<sub>0–∞</sub>).

## Statistical Analysis

Data are presented as mean  $\pm$  standard deviation (SD) and were analyzed using one-way ANOVA with extended LSD post hoc tests (subsequent multiple comparisons using Tukey's test), except for T<sub>max</sub> data which were analyzed by non-parametric Kruskal–Wallis test. All statistical tests were performed using IBM SPSS Statistics version 23, 64-bit edition, NY, USA. A P value of less than 0.05 was considered statistically significant.

## Results and Discussion

### Analysis of Factorial Design

The effective ranges of the independent variables were determined after performing preliminary trials (data not shown). Four independent variables were chosen: SAA type (X1), PC: SAA ratio (X2), cholesterol: SAA ratio (X3), and ethanol percent concentration (X4). EE% (Y1), PS (Y2), PDI (Y3), and ZP (Y4) as dependent variables were measured to choose the best three formulae solutions (Figure 1A–F).

### The Effect of Formulation Variables on EE %

Incorporation of drugs into phospholipid formulations enhances its delivery, stability, provide protection, and increase permeability, depending on lipid properties and compositions.<sup>30</sup> The effect of the independent variables, SAA type (X1), PC: SAA ratio (X2), cholesterol: SAA ratio (X3) and concentration of ethanol % (X4) on the EE % in drug vesicles is obtained in Table 2.

SAA type, PC: SAA ratio and presence of additives had no significant effect on EE %.

The concentration of ethanol % (X4) had a significant effect on EE % ( $P = 0.0176$ ). Hydration media with an ethanol concentration of 5% showed higher entrapment efficiency.

The relatively high entrapment efficiency may be due to the multi-lamellarity of ethosomal vesicles. The entrapment efficiency depends on ethanol concentration, entrapment efficiency increased by increasing ethanol concentration to a certain limit, after which the vesicle membrane became more permeable, so entrapment efficiency decreased.<sup>31</sup>

### The Effect of Formulation Variables on PDI

The effect of the independent variables, SAA type (X1), PC: SAA ratio (X2), cholesterol: SAA ratio (X3), and concentration of ethanol % (X4) on the PDI in drug vesicles is obtained in Table 2.

The smaller the PDI (lower than 0.7),<sup>32</sup> the more homogenous and uniform the dispersion is.

SAA type, PC: SAA ratio and concentration of ethanol % had no significant effect on PDI.

The presence of additives (X3) in the formulae had a significant effect on PDI ( $P = 0.0239$ ).

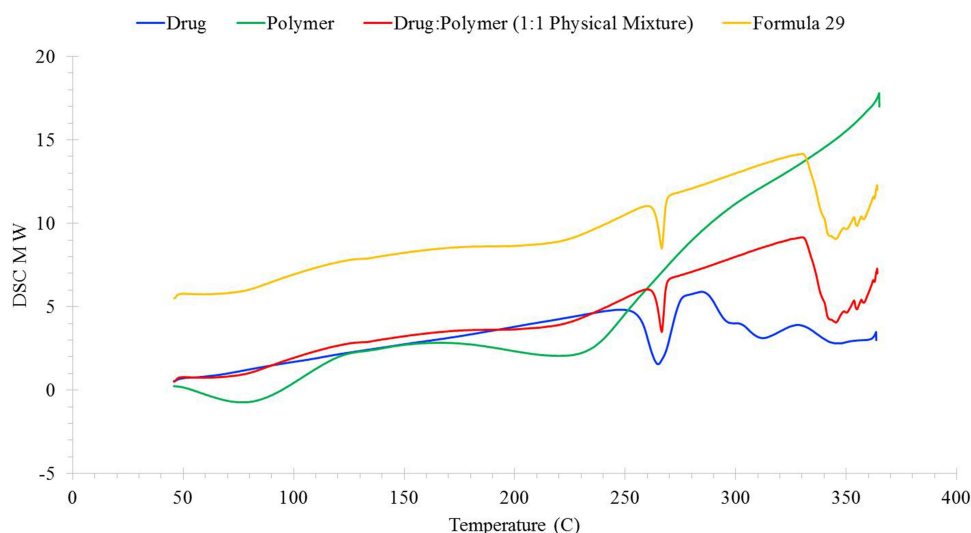
Presence of cholesterol: SAA (2:1) showed lower PDI; it might be due to the cholesterol features that decrease vesicles' bilayer leakage and produce surface smoothness. With a high

Table 2 Experimental Runs, Independent Variables, and Measured Response of the Selected Three Formulae of TEL-Loaded TEs

TE Formulation	X1	X2	X3	X4	Y1	Y2	Y3	Y4	Desirability
	Conc. of Ethanol %	Type of SAA	Additives	PC: SAA Ratio	EE%	PS (nm)	PDI	ZP (mV)	
TE29	5	Span 60	Cholesterol:SAA (2:1)	95:5	97% ±0.26	244 ±5.88	0.57 ±1.9	-31.6 ±1.59	0.831
TE31	5	Span 60	Cholesterol:SAA (2:1)	85:15	89% ±0.25	337 ±4.6	0.5 ±1.4	-28.3 ±3.79	0.811
TE32	5	Na deoxycholate	Cholesterol:SAA (1:1)	75:25	88% ±0.17	382.2 ±3.06	0.63 ±2.2	-31 ±2.65	0.798

Note: Data represented as mean ± SD.

Abbreviations: EE%, entrapment efficiency percentage; TEL, telmisartan; PC, phospholipid; PDI, polydispersity index; PS, particle size; S60, Span 60; SAA, surfactant; SDC, sodium deoxycholate; T80, Tween 80; TE, transthesosome; ZP, zeta potential.



**Figure 2** DSC Thermogram of drug (Telmisartan), polymer (Carbopol 940), drug to polymer physical mixture and formula 29

cholesterol level, this effect has been diminished as it introduces crystallinity to the bilayers.<sup>33</sup>

## The Effect of Formulation Variables on Zeta Potential

PC: SAA ratio and presence of additives had no significant effect on ZP.

The SAA type (X1) had a significant effect on ZP ( $P = 0.0002$ ). Sodium deoxycholate showed the highest ZP value (as absolute value); It might be due to the presence of 2  $\text{OH}^-$  groups that increase the negative value of ZP that prevents aggregation of vesicles.<sup>34</sup> The concentration of ethanol % (X4) had a significant effect on ZP ( $P = 0.0309$ ). Hydration media with an ethanol concentration of 20% showed the highest ZP value (as absolute value).

## The Effect of Formulation Variables on Particle Size

Due to different formulae components and ratios, their particle size varied between the lowest and the highest values. The lowest Ps was shown in F16, F29, and F31 at approximately (172.2, 244, and 337 nm respectively), while the highest value was shown in F5 at approximately (2066 nm). The statistical analysis showed non-significance of any of the four tested variables on the PS results using design Expert.

## Selection of the Optimum Formula

Certain criteria were set in Design-Expert software version 11 to select the best formula. These criteria preferred particles

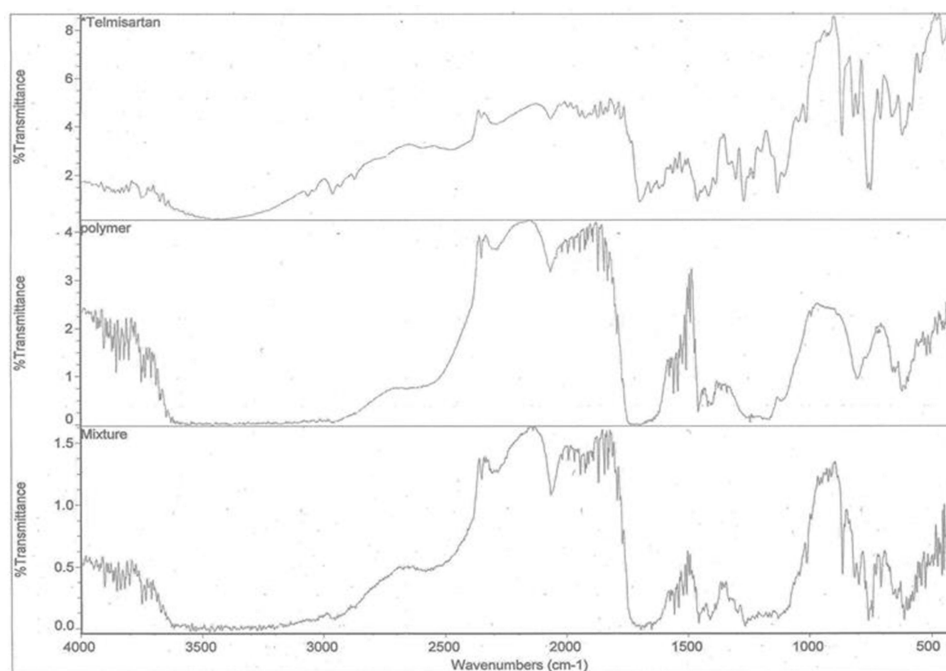
with the highest ZP (as an absolute value), EE%, and the lowest PDI and PS. The optimum three formulae were (F29, F31, F32) which met these specifications. F29 was composed of S60 as SAA, PC: SAA ratio of 95:5, ethanol concentration of 5% and cholesterol: SAA ratio of 2:1. TE29 showed an EE% of  $97\% \pm 0.26\%$ , a PS of  $244 \pm 5.88$  nm, a PDI of  $0.57 \pm 1.9$ , and ZP of  $-31.6 \pm 1.59$  mV. F31 composed of S60 as SAA, PC: SAA ratio of 85:15, ethanol concentration of 5% and cholesterol: SAA ratio of 2:1. TE31 showed an EE% of  $89\% \pm 0.25\%$ , a PS of  $337 \pm 4.6$  nm, a PDI of  $0.5 \pm 1.4$ , and a ZP of  $-28.3 \pm 3.79$  mV. F32 composed of SDC as SAA, PC: SAA ratio of 75:25, ethanol concentration of 5% and cholesterol: SAA ratio of 1:1. TE29 showed an EE% of  $88\% \pm 0.17\%$ , a PS of  $382.2 \pm 3.06$  nm, a PDI of  $0.63 \pm 2.2$ , and ZP of  $-31 \pm 2.65$  mV. The predicted and observed responses of these formulae were compared to validate this experiment; a high correlation was observed between the predicted and actual values (Table 2).

## Differential Scanning Calorimetry (DSC)

A sharp endothermic crest was obtained at  $264.93^\circ\text{C}$  with the heat of enthalpy of  $-65.54$  joule/gm. No sharp peak was observed at carbopol 940 thermogram. The physical mixture of telmisartan with carbopol 940 and formula 29 showed the characteristic peak of telmisartan at  $264.93^\circ\text{C}$ , indicating that, no interactions between the mixture components (Figure 2).

## Fourier Transformed Infrared Spectroscopy (FTIR)

The IR spectra of Telmisartan, Carbopol 940, and a physical mixture of Telmisartan and Carbopol 940 are illustrated in



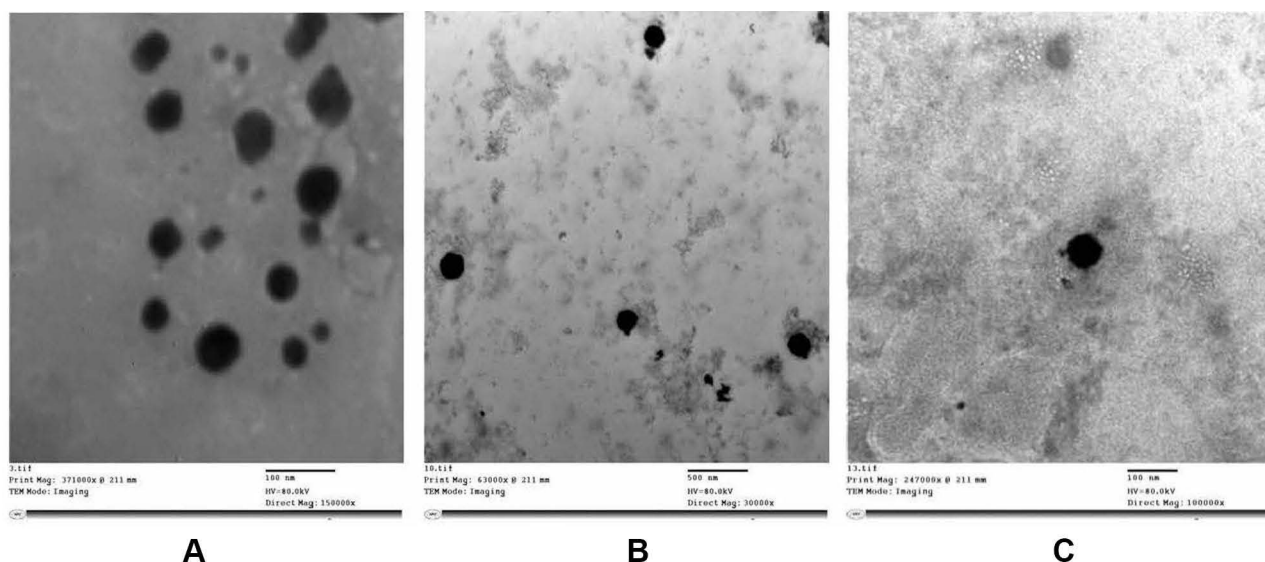
**Figure 3** Fourier transform infrared (FT-IR) spectra of drug (Telmisartan), polymer (Carbopol 940) and drug& polymer physical mixture

**Figure 3.** For telmisartan, a broad band at 3500–2500 $\text{cm}^{-1}$  was obtained for the OH group of the COOH group, a strong sharp peak at 1695 $\text{cm}^{-1}$  for the C=O group, and 3 peaks for aromatic C=C stretching at 1600  $\text{cm}^{-1}$ , 1420  $\text{cm}^{-1}$ , 1415  $\text{cm}^{-1}$ , and two peaks for  $sp^3\text{C-H}$  at 2919  $\text{cm}^{-1}$  and 2850  $\text{cm}^{-1}$ . The mixture showed the characteristic peaks for Telmisartan in the range of 3500–2500  $\text{cm}^{-1}$  for the OH band of the COOH group, a strong sharp peak at 1730  $\text{cm}^{-1}$  for the

C=O group, peaks for aromatic C=C stretching at 1460  $\text{cm}^{-1}$ , and peaks at 2924 and 2855  $\text{cm}^{-1}$  representing  $sp^3\text{CH}$ . This indicates that Telmisartan had no interaction with carbopol 940.

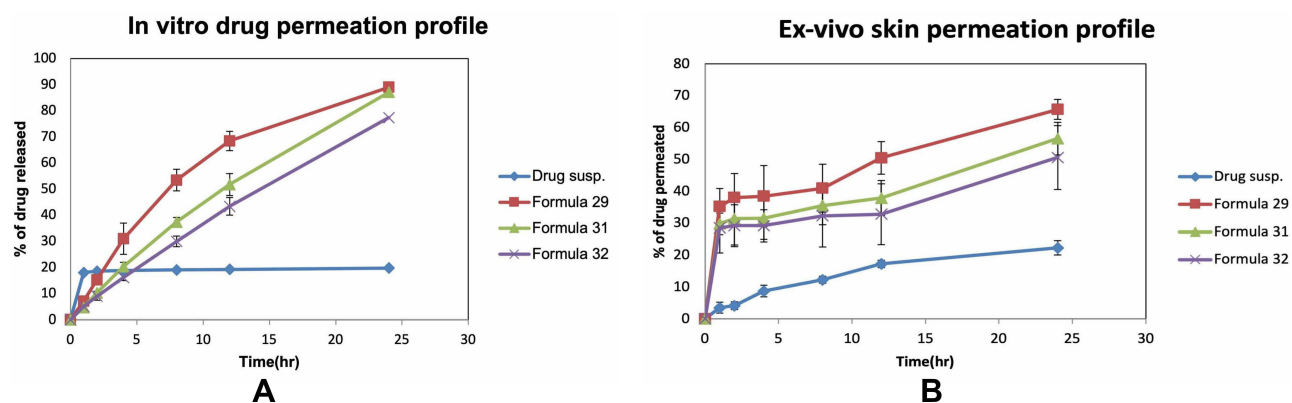
### Transmission Electron Microscopy (TEM)

TEM analysis was used to determine the external morphology of the optimum formulae (TE29, 31, 32). The analysis



**Figure 4** (Abbreviation TE, transethosome), **A** Transmission Electron Micrograph of TE29; **B** Transmission Electron Micrograph of TE31; **C** Transmission Electron Micrograph of TE32





**Figure 5 A** In vitro drug release of transthesosome different formulae vs drug suspension (mean  $\pm$ SD, n=3); **B** Ex vivo drug permeation of transthesosome different formulae vs drug suspension (mean  $\pm$  SD, n=3).

showed that the vesicles' morphology was spherical with a uniform size distribution (Figure 4). The PS results obtained from Zetasizer comply with TEM results.

### In vitro Release of TEL from Ethosomal Gel

The dissolution of TEL from ethosomal gel (F29, F31, and F32) and telmisartan suspension were evaluated in phosphate buffer 7.4: ethanol (30:20). However, it was noticed that the amount of TEL permeated through the membrane in the same order: (F29 > F31 > F32 > TEL susp.) (Figure 5A). TEL suspension can be best described as uncontrolled and poor release as the total amount dissolved at the end of 24 hours was only 20% and most of this quantity was released at the first hour. The release of all ethosomal formulae did not exceed 35% during the first hour, indicating a controlled release manner. The release of all ethosomal formulae showed a controlled release of TEL during the 24 hours of the experiment from the start to the end. At the end of the release, F 29, F31, and F32 showed approximately 89%, 87%, and 77% drug release, respectively, while the drug susp release showed only 20%.

### Ex vivo Drug Permeation of Telmisartan

To simulate the human skin, a wide range of animal models have been suggested and used to evaluate the percutaneous permeation of drugs. A full thickness rat skin, consisting of SC and viable epidermis and dermis

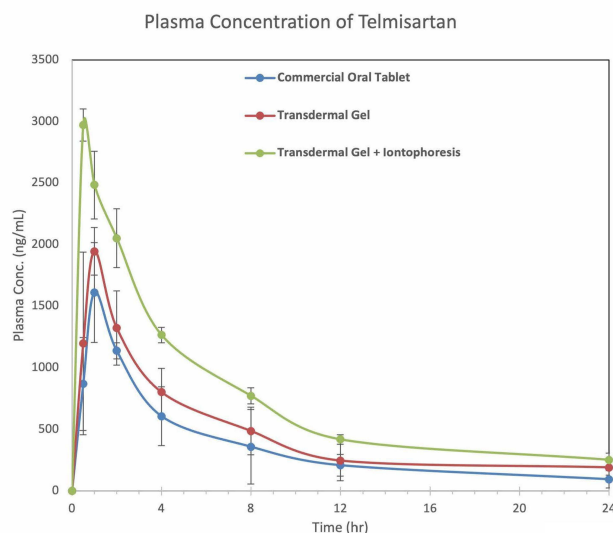
situated below the stratum corneum, was used for ex vivo drug permeation studies.<sup>35</sup> % of drug permeated from the formulae F29, F31, and F32 was significantly higher than the % permeated from TEL suspension ( $P \leq 0.05$ ) in the same order (F29 > F31 > F32 > TEL susp.) as shown in Figure 5B. The mechanisms of enhanced dissolution rate of TEL from formulae can be ascribed to several factors, such as the reduction of particle size, the reduction in interfacial tension between hydrophobic drug and dissolution medium, and improved wettability.<sup>36</sup> The optimum formula (F29) was selected for the in vivo study.

### In vivo Evaluation of Telmisartan Ethosomal Gel

The pharmacokinetic study is performed to determine the amount of drug permeated through rat skin. The amount of TEL absorbed from a commercial oral drug, F29 gel, and F29 gel with iontophoresis (Figure 6A) at different time intervals is shown in Figure 6B. The skin after applying F29 ethosomal gel with iontophoresis showed a significantly higher Cmax of  $2971.5 \pm 130.66$  ng/mL and Tmax of 0.5hrs in the skin layers when compared to formula gel with a Cmax of  $1944.438 \pm 193.58$  ng/mL and commercial oral drug with a Cmax of  $1611.36 \pm 405.58$  ng/mL both with Tmax of an hour ( $P \leq 0.05$ ). The use of iontophoresis with transdermal gel increased the transdermal penetration of telmisartan, thus it appeared in circulation with



A



B

**Figure 6** A Setting of experiment of application of Telmisartan transethosome gel Iontophoresis; B Comparative study of plasma concentration-time curve for commercial product, formula 29 and formula 29 with Iontophoresis.

a greater amount (higher  $C_{max}$  and AUC) with a rapid effect ( $T_{max}$  of 0.5hr) (Table 3).

## Conclusion

Thirty-two formulae of telmisartan ethosomes were prepared using the thin-film hydration technique. The best three formulae (F29, F31, and F32) were selected based on their desirability factor and were formulated into a gel. F29 showed statistically significant higher

telmisartan release through the dialysis bag and through rat skin (ex vivo study), so it was selected for the in vivo study. The in vivo comparative pharmacokinetics study of the prepared gel (Formula 29 augmented by Iontophoresis) compared to the commercial oral tablet resulted in higher Telmisartan  $C_{max}$  using Iontophoresis than both ethosomes transdermal gel alone and the commercial oral tablet and lower  $T_{max}$  as well. These results confirmed that combining

**Table 3** Pharmacokinetic Parameters After Administration of TEL Oral Commercial Tablet, TEL Transethosome-Loaded Gel, and TEL Transethosome-Loaded Gel with I Iontophoresis

PK Parameters	Treatment		
	Commercial Oral Tablet	Transdermal Gel	Transdermal Gel + Iontophoresis
$C_{max}$ (ng/mL)	1611.36±405.5	1944.43±193.5	2971.52±130
AUC <sub>0-24</sub> (hr*ng/mL)	8853.36±3642.7	11519.13±4002.5	18179.26±280.9
AUC <sub>0-∞</sub> (hr*ng/mL)	9915.46±4348.8	14112.79±5358.5	20972.24±137.5
$T_{max}$ (hr)	1	1	0.5
AUMC <sub>0-24</sub> (hr*hr*ng/mL)	57414.14±31778.5	82204.77±34552.4	125270.62±2121.5
AUMC <sub>0-∞</sub> (hr*hr*ng/mL)	94818.77±55583.9	180265±91957.2	223223.03±14830.6
$T_{1/2}$ (hr)	8.072±0.8	9.074±1.9	7.71±0.5
MRT (hr)	6.278±1.006	7.04±0.553	6.89±0.2
$\lambda_{z}$ (1/hr)	0.086±0.01	0.078±0.016	0.09±0.006

**Note:** Data represented as mean ± SD.

**Abbreviations:**  $C_{max}$ , maximum plasma concentration; AUC, area under the curve;  $T_{max}$ , time required to reach the maximum plasma concentration; AUMC, area under the first moment curve;  $T_{1/2}$ , time required to reach 50% of the plasma concentration; MRT, mean residence time;  $\lambda_{z}$ , terminal elimination rate constant.

ethosomal nanovesicles with iontophoresis provides a promising method for delivering Telmisartan transdermally.

## Disclosure

The authors report no potential conflicts of interest in this work.

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