

Original Article

## ENHANCED TRANSDERMAL PERMEABILITY OF TELMISARTAN BY A NOVEL NANOEMULSION GEL

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### ABSTRACT

**Objective:** Telmisartan is an angiotensin II type I receptor blocker antihypertensive agent with 42% oral bioavailability. The aim of the present investigation was to develop a nanoemulsion gel to enhance bioavailability of poorly water soluble Telmisartan.

**Methods:** Different nanoemulsion components (oil, surfactant and co-surfactant) were selected on the basis of solubility and emulsification ability. Pseudoternary phase diagrams were constructed using aqueous titration method. Carbopol 934 was added as a gel matrix to convert nanoemulsion into nanoemulsion gel. Drug loaded nanoemulsions and nanoemulsion gels were characterized for particle size, viscosity, rheological behavior, thermodynamic stability studies and *ex vivo* permeation studies using rat skin. Transdermal permeation of Telmisartan from nanoemulsion gels was determined using Franz Diffusion cell.

**Results:** The optimized nanoemulsion gel (NEG) contained Labrafil®M 2125 CS (14.3%) as oil, Acrysol®EL 135 (30.84%) as surfactant, Carbitol® (15.42%) as co-surfactant and (32.44%) water; 20 mg drug and 1% w/w carbopol. The *ex vivo* permeation profile of optimized formulation was compared to nanoemulsion and normal gel. Permeability parameters like steady-state flux (J<sub>ss</sub>), permeability coefficient (K<sub>p</sub>), and enhancement ratio (Er) were significantly increased in nanoemulsion (NE) and nanoemulsion gel (NEG) as compared to conventional gel. There was a considerable improvement in bio availability for nanoemulsion gel compared to the conventional telmisartan gel.

**Conclusion:** Nanoemulsion gel has significantly increased the bio-availability of the drug.

**Keywords:** Telmisartan, Nanoemulsion gel, Topical delivery, Phase diagram.

### INTRODUCTION

Telmisartan [2-(4-{ [ 4 - methyl - 6 -(1 - methyl-1H -1, 3 -benzodiazol -2-yl)-2-propyl-1H-1, 3-benzodiazol-1-yl ]methyl} phenyl) benzoic acid ], is an angiotensin II receptor blocker, which displaces angiotensin II from the angiotensin I receptor and produces the blood pressure- lowering effects by antagonizing angiotensin II-induced vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake and hypertrophic response. Telmisartan is practically insoluble in water (0.0035 mg/ml) and is highly hydrophobic (log P 6.66) with only 42% oral bioavailability [1]. A lipid-based drug delivery system is ideal for improving the bio availability of such poor water soluble drug [2]. Hence, Telmisartan was selected as a model drug for this study. Telmisartan is available in various doses (20 mg, 40 mg, 80 mg and 120 mg); 20-mg dose was selected as a working dose for the present study.

The term "Nanoemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water stabilized by an interfacial film of surfactant and co-surfactant molecules having droplet size less than 100 nm [3]. The attraction of formulating nanoemulsion systems lies in their ability to incorporate hydrophobic drugs into the oil phase, thereby enhancing their solubility [4]. Several mechanisms have been proposed to explain the advantages of nanoemulsion for the transdermal delivery of drugs. First, the high solubilising potential for drugs of nanoemulsion system might increase the thermodynamic activity towards the skin [5]. Second, ingredients of nanoemulsion, acting as permeation enhancers, might increase the flux of drug via skin [6]. Third, the permeation rate of the drug from nanoemulsion may be increased because the affinity of a drug to the internal phase could be modified easily to favor partitioning into stratum corneum [7]: Since nanoemulsions contain surfactant compounds in its composition, the application to the skin surface usually produces an increase in the membrane permeability facilitating transdermal transport [8]. The literature shows that nanoemulsion can control release and improve bioavailability of many drug compounds [9]. In this study, an optimum topical nanoemulsion gel containing Telmisartan was developed after screening various oils to improve the drug solubility and skin permeability of the drug.

### MATERIALS AND METHODS

#### Materials

Telmisartan was obtained as a gift sample from NMR labs, Hyderabad, Telangana, India. Labrafil M 2125 CS (Linoleoyl macroglycerides), Plurol Oleique® (Polyglycerololeate), and Capryol®90 (Polypropylene glycol monocaprylate) were received as a gift sample from Gattefosse, Mumbai, Maharashtra, India. Cremophor® EL (Polyoxyl 35 hydrogenated castor oil) and Cremophor® RH 40 (Polyoxyl 40 hydrogenated castor oil) were a kind gift by Croda Chemicals Private Ltd., Mumbai, Maharashtra. Capmul®MCM (Glycerol mono- dicaprylate) and Capmul® GMO (Glycerol mono/di-oleate) were received as gift samples from Abitec, India. Acrysol® K 140 (polyoxyl 40 hydrogenated castor oil) and Acrysol® EL -135 (polyoxyl 35 castor oil) were also gifted from Corel Pharma, Gujarat, India. Other chemicals like Oleic acid, Iso-propyl myristate, Ethanol, Propylene glycol, Span® 20 (sorbitan-monolaurate), Span® 80 (sorbitan-monooleate), Tween®20 (polyoxyethylene sorbitan monolaurate), Tween®80 (polyoxyethylene sorbitan monooleate), Polyethylene glycol 400 (PEG 400), polyethylene glycol 600 (PEG 600), Glycerol, Olive oil, Linseed oil, Castor oil and Methanol was purchased from Merck India. Carbopol®934 and Carbopol®940 were obtained as gift samples from Loba Chemie, Mumbai, Maharashtra, India. Transcutol®P (Monoethyl ether of diethylene glycol) was purchased from Avra Laboratories Pvt. Ltd., Hyderabad, India. Double distilled water was used throughout the study and all other reagents used were of analytical grade.

#### Methods

##### Selection of nanoemulsion components

##### Solubility studies

The solubility of Telmisartan in oils, surfactants, and co-surfactants was measured using the shake flask method. An excess amount of Telmisartan was introduced into each excipient (2 ml) followed by sealing in vials and stirred for 72h at 30°C using a table top orbital Shaker (Eltek®India) to facilitate the solubilisation. Each vial was

centrifuged at 15,000 rpm for 10 minutes using a research centrifuge (REMI) followed by the removal of undissolved Telmisartan by filtering through a membrane filter (0.45 µm\*). Samples were suitably diluted with methanol and a drug concentration was obtained by using a double-beam UV visible spectrophotometer (Lab India UV 3000+) at 296 nm using methanol as a blank ( $R^2 = 0.9998$ , linearity 2-14 µg/ml). The experiment was repeated thrice. The results were represented as mean values (mg/ml±SD) [10] via a validated UV method

#### Surfactant (Emulsification study)

Different surfactants (Acrysol® EL 135, Tween® 20, and Tween®80) were screened for the emulsification ability of the selected oil phase. Surfactant selection was done on the basis of percentage transparency and ease of emulsification. Briefly, 300 mg of the surfactants was added to 300 mg of the selected oily phase. The mixtures were gently heated at 50°C for the homogenization of the components. Each mixture, 50 mg, was then diluted with distilled water to 50 ml\* in a stoppered conical flask. Ease of emulsification was judged by the number of flask inversions required to yield a homogenous emulsion. Emulsions were allowed to stand for 2 h and their percentage transparency was evaluated at 560 nm by a double-beam UV spectrophotometer using distilled water as a blank. Emulsions were furthermore observed visually for any turbidity or phase separation.

#### Co-surfactant (Emulsification study)

Three co-surfactants were screened for nanoemulsion formulation, which included Carbitol®, PEG 600 and Ethanol. The screening of the co-surfactant was conducted on the basis of percentage transparency and ease of emulsification. Mixtures of 100 mg of the co-surfactant, 200 mg\* of the selected surfactants and 300 mg of the selected oil were prepared and evaluated for percentage transparency at 560 nm [10].

#### Construction of the ternary phase diagrams

On the basis of solubility and emulsification study Labrafil®M 2125 CS, Acrysol®EL 135 and Carbitol® were selected as oil, surfactant and co-surfactant, respectively. To determine the concentration of components in the existing range of the nanoemulsion, a pseudo-ternary phase diagram was constructed using an aqueous titration method at ambient temperature (25°C). The surfactant and co-surfactant were mixed in different volume ratios (1:1, 1:2, 1:3, 1:4, 4:1, 3:1 and 2:1). Oil and surfactant / co-surfactant ( $S_{mix}$ ) were mixed thoroughly in different volume ratios (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) and titrated with water by drop wise addition under gentle agitation. Slow titration with aqueous phase was done to each weight ratio of oil and  $S_{mix}$  and visual observation was carried out for transparent and easily flowable o/w nanoemulsions. The proper ratio of one excipient to another in the nanoemulsion formulation was analyzed and the pseudo-ternary plot was constructed using TRIPLLOT V14 software (version 4.1.0.2). All studies were repeated three times, with similar observations being made between repeats.

#### Thermodynamic stability studies

1. Heating cooling cycle: Six Cycles between refrigerator temperature (4 °C) and (45°C) with storage at each temperature for not less than 48 h was studied. Formulations, which were stable at these temperatures were subjected to centrifugation test.
2. Centrifugation: Stable formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.
3. Freeze thaw cycle: Three freeze thaw cycles between -21°C and 25°C with storage at each temperature for not less than 48 h was done for the formulations.

The formulations that passed the thermodynamic stability tests were selected for further studies.

#### Preparation of Telmisartan nanoemulsion

Telmisartan (20 mg) was added in accurately weighed amounts of oil into a beaker. The surfactant and co-surfactant were added to the

oil mixture using a positive displacement pipette and magnetically stirred. The formulations were further sonicated (Sonica ultrasonic, 2000 MH, Spinotech Pvt Ltd, India) for 15 minutes and stored at room temperature until their use in subsequent studies.

#### Evaluation parameters of Telmisartan nanoemulsion

##### *In vitro* drug release studies

*In-vitro* drug release of Telmisartan from the nanoemulsion formulation was determined using locally fabricated Franz diffusion cell. Nanoemulsion equivalent to single dose (20 mg of Telmisartan) was placed in a donor compartment of diffusion cell. Receptor compartment was filled with pH 7.5 Phosphate buffer solution with 1% SLS and was stirred continuously at 350 rpm. The receptor and donor compartments were separated by Hi-media dialysis membrane 150 (molecular weight cut off 12000-14000 Dalton, pore size 0.4 nm). Samples were withdrawn at specific time intervals and an equal volume of medium was replaced to maintain sink condition. The samples were analyzed by the UV-Visible spectrophotometer at 298 nm to determine the concentration. The experiment was repeated thrice. The results were represented as mean values (Percentage Cumulative Drug Release) (%CDR±SD). Formulation having highest %CDR was considered as optimized formulation and used for subsequent studies.

##### Globule size and zeta potential determination

50 mg of the optimized nanoemulsion formulation was diluted with water to 100 ml in a flask, and gently mixed by hand. The droplet size distribution and zeta potential of the resultant emulsion was determined by laser diffraction analysis using a particle size analyzer (Horiba Scientific nanopartica, UK), that analyzes the fluctuations in light scattering due to Brownian motion of the particles. Light scattering was monitored at 25°C at a 90° angle.

##### Percent transmittance

The percent transmittance of the nanoemulsion was measured using UV-Visible double beam spectrophotometer using distilled water as blank at 560 nm.

##### Viscosity

The viscosity of the samples was measured as such without dilution using a Brookfield viscometer (LVDV-II+P) fitted with an S-34 spindle at 25°C. Studies were repeated three times and results are described in mean±SD.

#### Preparation of Telmisartan nanoemulsion gel and Telmisartan gel

Carbopol 934 (1% w/w) was dispersed in sufficient quantity of distilled water and added to optimized nanoemulsion. The mixture was stirred for a few minutes and glycerol (10%) was added. The gel was then neutralized with 1% Triethanolamine in water. Telmisartan loaded gel (TG) was prepared by dispersing Carbopol 934 (1 % w/w) in a mixture of distilled water and glycerol (10%) with 20 mg\* telmisartan, the mixture was magnetically stirred until a homogeneous dispersion formed. The dispersion was then made viscous and neutral by adding Triethanolamine. Prepared Telmisartan nanoemulsion gel (NEG) and Telmisartan loaded gels (TG) were stored at room temperature until their use in subsequent studies.

#### Evaluation parameters of Telmisartan nanoemulsion gel

##### pH

The pH of aqueous solutions (1%) of the gel is measured by a pH meter (Systronics, µ pH System 361) at 25°C±1°C. Studies were repeated thrice and results are described in mean±SD.

##### Drug content

Quantity of Telmisartan in nanoemulsion gel was determined by UV-Spectrophotometer. 1.0 gm\* formulation was accurately weighed, dissolved in 50 ml\* of methanol, filtered and diluted if required. Absorbance was determined using a UV spectrophotometer. Studies were repeated thrice and results are described in mean±SD.

### Drug-Excipient compatibility studies

Fourier transform infrared analysis (SHIMADZU) was conducted to study the drug excipient interactions. Samples were scanned in the range from 400-4000  $\text{cm}^{-1}$ . The drug excipients compatibility study was determined using Potassium bromide (KBr) pellet method and scanned in the range of 4000  $\text{cm}^{-1}$  to 400  $\text{cm}^{-1}$ . The IR spectrum of the pure drug was compared to IR spectrum of optimized formulation to check for interaction.

### Ex-vivo skin permeation study

Abdominal skin was excised from experimental rat, whose hair had been previously removed. Subcutaneous fat and other visceral tissue was removed carefully. Franz diffusion cells with an effective diffusion area of 2.0  $\text{cm}^2$  with the diameter of 16 mm and receptor volume of 12.5 ml were used to assess *in vitro* drug permeation. Donor and receptor compartments were separated by freshly excised rat skin. The receptor compartment was kept at 37°C filled with receptor fluid with pH 7.5 phosphate buffer with 1% SLS. The hydrodynamics in the receptor compartment were maintained by stirring continuously with magnetic stirrer at 500 rpm. Each formulation equivalent to single dose was placed in the donor compartment. Permeation experiments were carried out for 24 h after application. Samples were withdrawn from the receptor compartment at scheduled time intervals and immediately replaced with the same volume of fresh receptor fluid. The amount of Telmisartan in the samples was determined by UV-visible spectrophotometer at 296 nm using freshly prepared pH 7.5 phosphate buffer with 1% SLS as blank. Studies were repeated thrice and results are described in mean  $\pm$  SD.

### Permeation data analysis

The permeation profiles were constructed by plotting the cumulative amount of Telmisartan permeated per unit rat skin area ( $\mu\text{g}/\text{cm}^2$ ) versus time. Linear regression analysis was used to calculate the steady state flux ( $J_{ss}$   $\mu\text{g}/\text{cm}^2$  / hr) of Telmisartan by using the slope of the plot. The following equation was used to determine the permeability coefficient ( $K_p$ ) of the drug through the stratum corneum:

$$K_p = J_{ss} / C$$

Where, C is the initial concentration of the drug in the donor compartment. The penetration enhancing effect was calculated in terms of enhancement ratio (Er) by using the following equation:

$$E_r = \frac{J_{ss} \text{ of Nanoemulsion gel formulation}}{J_{ss} \text{ of control formulation}}$$

All data are shown as mean  $\pm$  standard deviation.

### In vivo studies

The studies were performed as per the guidelines of the institutional animal ethics committee (No. 1722/PO/A/13/IAEC/CPCSEA EXP-050). The rats were deprived of food but had free access to water 24 h before the day of the experiment. Two groups of rats were used for

the experiments. Each group was either administered Telmisartan normal gel (control group) or Telmisartan nanoemulsion gel topically under ether anesthesia, blood samples (0.5 ml) were collected via the retro-orbital vein at 2, 4, 6, 8, 10, 12 hours after topical administration into heparinised microcentrifuge tubes. The samples were centrifuged at 15,000 rpm for 10 min at 4°C temperature. The plasma samples (100  $\mu\text{l}$ ) were separated, and 1 ml\* of acetonitrile was added to each of the plasma samples to precipitate the protein. The samples were then centrifuged again at 15,000 rpm, 4°C for 5 min, and the supernatant (20  $\mu\text{l}$ ) was directly injected on to the HPLC (Waters.) Chromatographic column (Aquity) C8 (150 cm and 4.6 mm id.) with a 5  $\mu\text{m}$  particle size was used Acetonitrile and methanol (55:45) were utilized as a mobile phase at a flow rate of 1.0 ml/min with total run time of 10 min. Data from these samples were used to plot curves for telmisartan absorption with time.

### Pharmacokinetic parameters

Winnonlin software (5.3 version) was used to estimate the peak plasma concentration ( $C_{max}$ ), the time to reach  $C_{max}$  ( $t_{max}$ ), total area under the curve ( $AUC_{0 \text{ to } \infty}$ )

## RESULTS AND DISCUSSION

### Selection of components

#### Solubility studies

The drug should have good solubility in components of the nanoemulsion. This is to prevent precipitation of the drug during shelf life of the formulation and after dilution in G. I. fluids.

Nanoemulsion were prepared using different oils, surfactants and cosurfactants. The components of the formulation were selected based on solubility studies. The most important criterion for screening of excipients is the ability of oil, surfactants and co-surfactants to solubilise the poorly soluble drug. The solubility of Telmisartan in different oils, surfactants and cosurfactants was determined.

As described in table 1, the solubility of Telmisartan was found to be highest in Labrafil<sup>®</sup>M 2125 CS as compared to other oils. Hence, Labrafil<sup>®</sup>M 2125 CS was selected as the oil phase. The drug was found to be more soluble in Acrysol<sup>®</sup>EL 135, Span<sup>®</sup>80, Tween<sup>®</sup>20 and Tween<sup>®</sup>80 among surfactants and in ethanol, Carbitol<sup>®</sup> and PEG 600 among co-surfactants.

All fatty acids contain carbon chains with more than 12 carbon atoms because of which the formed emulsion containing telmisartan can direct the drug towards lymphatic system and can bypass hepatic metabolism of the drug.

Important factor for performance of SEDDS is the polarity of oil droplets. The polarity is governed by HLB, chain length and degree of unsaturation of fatty acid. Labrafil<sup>®</sup>M 2125 CS reduces o/w interfacial tension. It contains mono and di-glycerides of fatty acids and sometimes PEG esters which act as lipophilic and hydrophilic surfactant molecules respectively.

Table 1: Solubility study of Telmisartan in various excipients at 25°C

Oil	Solubility <sup>a</sup> in mg/ml $\pm$ SD	Surfactant	Solubility <sup>a</sup> in mg/ml $\pm$ SD	Co-surfactant	Solubility <sup>a</sup> in mg/ml $\pm$ SD
Capmul MCM	0.71 $\pm$ 0.06	Acrysol EL 135	6.53 $\pm$ 0.240	Ethanol	1.18 $\pm$ 0.22
Capmul GMO 140	1.70 $\pm$ 0.19	Acrysol K	2.34 $\pm$ 0.25	Glycerol	0.06 $\pm$ 0.013
Capryol 90	1.03 $\pm$ 0.07	Span 20	0.91 $\pm$ 0.09	Propylene Glycol	0.35 $\pm$ 0.02
Castor oil	1.04 $\pm$ 0.07	Span 80	1.56 $\pm$ 0.18	PEG400	2.76 $\pm$ 0.26
IsoPropyl Myristate	0.09 $\pm$ 0.01	Tween 20	1.91 $\pm$ 0.16	PEG 600	2.58 $\pm$ 0.29
Linseed oil	1.87 $\pm$ 0.11	Tween 80	2.43 $\pm$ 0.2	Transcutol P	3.19 $\pm$ 0.24
Oleic acid	3.36 $\pm$ 0.31			Ethanol	1.18 $\pm$ 0.22
Olive oil	1.19 $\pm$ 0.24			Glycerol	0.06 $\pm$ 0.013
Labrafil M 2125 CS	2.10 $\pm$ 0.01				

[<sup>a</sup>Data are expressed as mg/ml  $\pm$  SD (n = 3)]

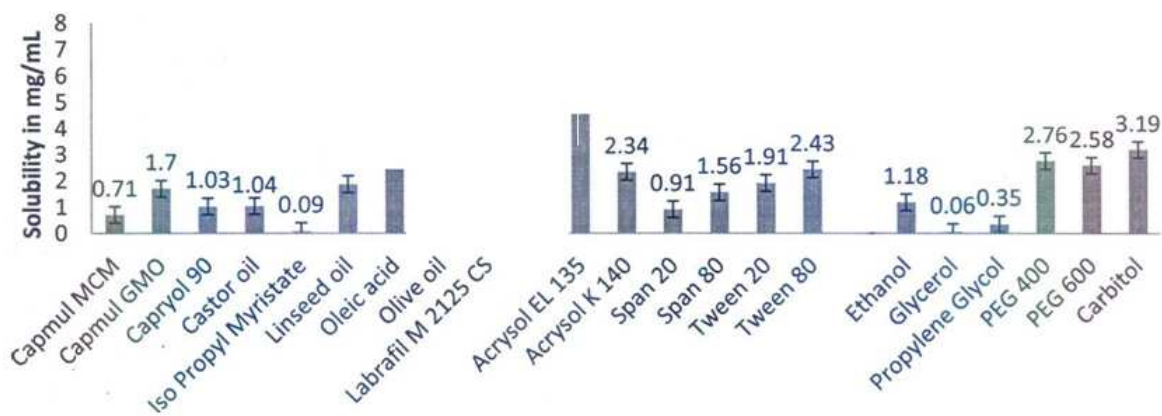


Fig. 1: Solubility study of Telmisartan in various excipients

### Preliminary screening of surfactants

The excipients selected were needed to be pharmaceutically acceptable, non-irritating and non-sensitizing to the skin and should fall into the GRAS (generally regarded as safe) category. Safety is a major factor in choosing a surfactant, as a large amount of surfactants may cause skin irritation [12]. Nonionic surfactants are considered to be less toxic than ionic surfactants and therefore Acrysol® EL 135, Span®80, Tween®20 and Tween®80 were selected, out of which some are reported to have bioactive effects, such as lymphotropic characters by Tween®20, Tween®80, and Span®80, and the inhibitory effect on P-gp and CYP enzymes by Acrysol® EL 135 [1].

Table 2: Emulsification efficiency of various surfactants

Surfactant	Number of inversions <sup>a</sup>	% transparency <sup>a</sup>
Acrysol EL135	7	93.50
Tween 20	18	89.69
Tween 80	37	76.89
Span 80	42	62.93

<sup>a</sup>Data expressed as mean (n=3)

Results inferred that the oily phase Labrafil® M 2125 CS exhibited the highest emulsification efficiency with Acrysol® EL 135 [% transparency: 93.5 %, 7 flask inversions] for the homogenous emulsion formation. On the other hand, Labrafil® M 2125 CS showed poor emulsification properties with other surfactants employed, requiring a higher number of flask inversions [Table 2]. The results suggested the use of Labrafil® M 2125 CS as an oily phase with Acrysol® EL 135 as a surfactant for further study.

### Preliminary screening of co-surfactants

The addition of a co-surfactant to the surfactant-containing formulation was reported to improve transparency and drug permeation from the formulation [1]. Additionally, co-surfactants decrease the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsion over a wide range of composition<sup>13</sup>. In the current investigation, two co-surfactants, namely, Carbitol® and PEG 600 were compared.

Table 3: Emulsification efficiency of various co-surfactants

Co-surfactant	Number of inversions <sup>a</sup>	% transparency <sup>a</sup>
PEG 600	5	95.32
Transcutol P	2	97.43

<sup>a</sup>Data expressed as mean (n=3)

As depicted in table 3, Labrafil® M 2125 CS exhibited good emulsification with both the 1 co-surfactants. Herein, the solubility of the drug in different co-surfactants may judge the final selection. The

results of the solubility study demonstrated in table 1 inferred a higher solubility in Carbitol®. It is worthy to note that all dispersions exhibited an instantaneous emulsion formation with only two flask inversions [table 3]. This could contend the importance of the co-surfactant addition to the-surfactant-containing dispersions.

### Construction of Pseudoternary phase diagram

The aim of the construction of the pseudoternary phase diagram was to find out the existing range of nanoemulsion. Care was taken to ensure that observations are not made on the metastable system [14]. Pseudoternary phase diagrams were constructed separately for each Smix ratio for getting o/w nanoemulsion regions. The area of nanoemulsion isotropic region changed slightly as the ratio of surfactant in Smix was increased. In the phase diagrams, the existence of large or small nanoemulsion region depends on the capability of the particular Smix to solubilise the oil phase. The extent of solubilisation results in a greater area with the formation of more clear and homogenous solution<sup>13</sup>. It is important to determine the optimum concentration of surfactant in a formulation because a high amount of surfactants can cause skin irritation [15].

Formulation of telmisartan nanoemulsion was planned in three groups:

- Labrafil® M 2125 CS as oil, Acrysol EL 135 as surfactant and Transcutol P as co-surfactant
- Labrafil® M 2125 CS as oil, Acrysol EL 135 as surfactant and Ethanol as co-surfactant
- Labrafil® M 2125 CS as oil, Acrysol EL 135 as surfactant and PEG 600 as co-surfactant

Among those three groups, group A formulations was successful, while group B and group C failed to form nanoemulsion. Group B and group C formulations became turbid and were not able to solubilise the drug completely. So, group B and group C were dropped for further study. Formulations prepared of Smix ratio 1:2 and 1:3, resulted in turbid nanoemulsion, as the drug was not completely solubilised. While in case of Smix 3:1 and 4:1, formulations were found to be highly Viscous. So, pseudoternary phase diagrams were constructed for group A with 1:1 and 2:1 Smix ratios.

### For different Smix ratios

A wider microemulsion region was obtained when Smix was incorporated in the ratio 2:1 (38%) when compared to Smix 1:1 ratio (26%).

### Thermodynamic stability studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. The formulations were subjected to thermodynamic stability studies which include heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Formulations, which survived thermodynamic stability tests were taken for further studies.

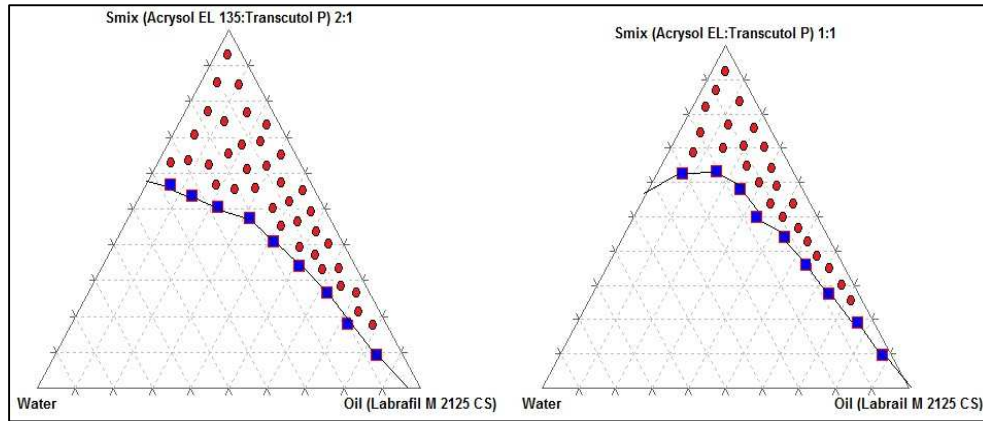


Fig. 2: Pseudoternary phase diagrams

Table 4: Thermodynamic stability studies

F-code	Percentage w/w of different components in formulation				Observation based on Thermodynamic stability studies			Inference
	Oil	S mix		Aqueous	H/C	Centri.	F/T	
		Sur	Co-sur					
NE1	19.69	39.37	39.37	1.57	✓	✓	✓	Passed
NE2	14.59	36.5	36.5	12.41	✓	✓	✓	Passed
NE3	12.26	36.81	36.81	14.11	✓	✓	✓	Passed
NE4	29.27	33.79	16.9	20.04	✓	✓	✓	Passed
NE5	19.30	32.17	16.09	32.44	✓	✓	✓	Passed
NE6	15.63	26.87	13.44	44.06	✓	✓	✓	Passed
NE7	13.33	26.66	26.66	29.41	-	-	-	Failed
NE8	11.79	35.37	17.68	35.14	-	-	-	Failed
NE9	15.31	30.62	30.62	23.43	-	-	-	Failed
NE10	13.35	40.05	20.02	26.56	-	-	-	Failed

Heating cooling cycle (H/C), centrifugation (Centri.), freeze thaw cycle (F/T), Formulation code (F-code), Surfactant (Sur), Cosurfactant (co-sur)

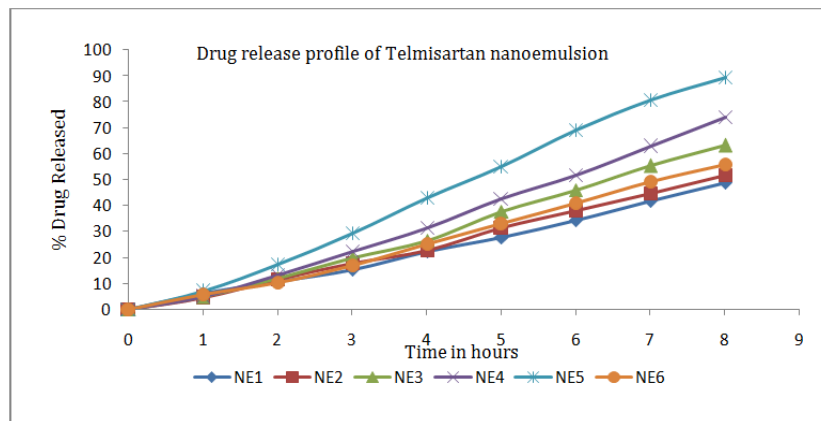


Fig. 3: In-vitro drug release for NE1 to NE6

**Evaluation parameters of Telmisartan nanoemulsion**

**In-vitro drug release studies**

In vitro studies were performed to compare the release rate of the drug from the various nanoemulsion formulations all having the same quantity of Telmisartan (20 mg). The release rate of NE5 (89.57%) was found to be the best as compared to NE4 (74%) and other formulations.

From the results of in vitro drug release and thermodynamic stability studies, Formulation

NE5 was optimized and can be carried for further studies.

**Globule size and zeta potential**

Globule size and zeta potential were measured using Horiba Scientific nanopartica, UK. Particle size of NE5 nanoemulsion formulation was found to be (50.9 nm±4.23 nm, mean±SD, n = 3). The Polydispersity index was found to be 0.311 which indicates a narrow size range as shown in fig. 4.

Zeta potential of NE5 nanoemulsion formulation was found to be (-32.5 mV±0.56 mV, mean±SD, n=3) which indicates that the particles of nanoemulsions are negatively charged and are stable as shown in fig. 5. The particles are in a de-flocculated and shall repel each other and impart physical stability to the system.



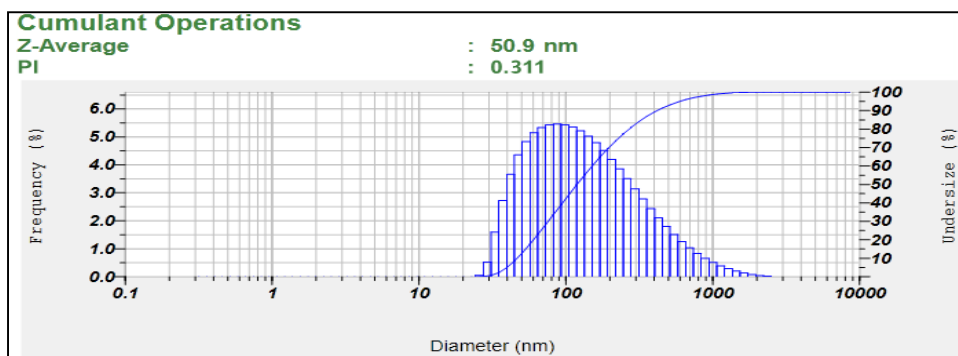


Fig. 4: Particle size of Telmisartan nanoemulsion

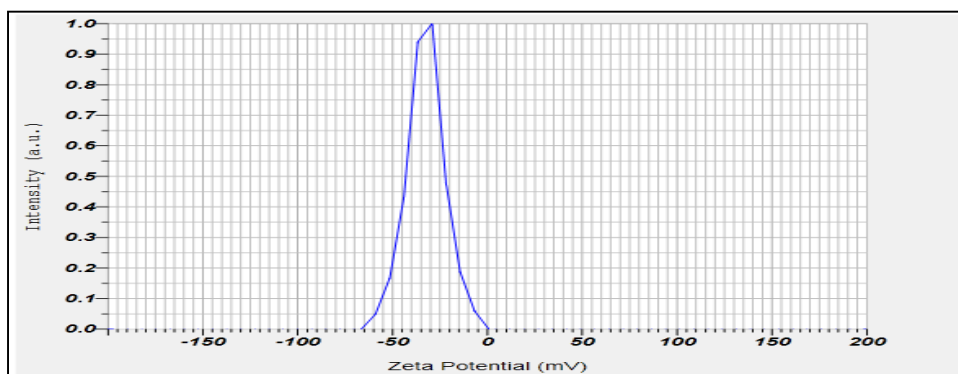


Fig. 5: Zeta potential of Telmisartan nanoemulsion

There are two factors that favor emulsion stability. These are relatively small volume of dispersed phase and narrow range of droplet size distribution PDI (0.311).

**Viscosity**

The viscosity of the nanoemulsion formulation NE5 was found to be (3.094 cP±0.68 cP, mean±SD, n = 3), as expected for o/w emulsion. It was not suitable for topical use, which justified, the incorporation of nanoemulsion into gel matrix, resulting in nanoemulsion gel having high value of viscosity.

**Percent Transmittance**

The percent transmittance of the NE5 formulations was measured at 560 nm\* using triple distilled water as a blank.

The percentage transmittance of the optimized formulation NE5 was found to be (93.3%±0.24%, mean± SD, n = 3). The results of percentage transmittance revealed that NE5 formulation was nearly transparent.

**Evaluation-parameters of Telmisartan nanoemulsion gel pH**

The pH of 1% aqueous solutions of the prepared nanoemulsion gel was found to be (6.93± 0.18, mean±SD, n = 3) at 25°C±1°C. Hence the preparation is non irritating to the skin.

**Drug content**

The drug content of the optimized formulation was found to be (96.13±1.95) % (mean±SD, n= 3).

**Drug- excipient compatibility studies**

FTIR spectrums are mainly used to determine if there is any interaction between the drug and any of the excipients used. The presence of interaction is detected by disappearance of important functional groups of the drug.

Telmisartan compatibility with the ingredients of nanoemulsion was tested using FTIR. The spectrum of telemisartan with the excipients had the feature of each of the components and did not

differ with the IR spectrum of the drug, this indicated that there is no interaction and that the molecular structure of telmisartan remained completely intact. Therefore the drug did not interact with any of the excipients of the formulation.

Table 5: Interpretation of FTIR Scan of drug

Functional Group	Frequency (1/cm)
O-H stretching	3700-2700
C=O stretching	1597.11

The characteristic peaks of the optimized formulation followed the same trajectory as that of the drug alone with minor differences (fig. 6 & fig. 7) thus, it can be concluded that there are no drug-excipient interactions

**Viscosity**

Viscosity of NEG-2 was determined using a Brookfield Viscometer, (DV-II+Pro.) Viscosity of the optimized gel is shown below:

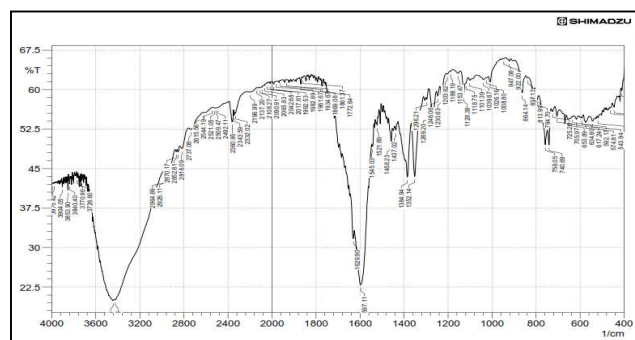


Fig.6: FTIR Spectrum of Telmisartan drug

From fig. 8, it can be inferred that the viscosity of the gels decreased with increasing shear rate due to their pseudoplastic behavior. This pseudoplasticity results from the colloidal network structure that aligns itself in the direction of shear, thereby decreasing the viscosity as the shear rate increases.

**Ex-vivo skin permeation study**

Ex-vivo skin permeation studies were performed to compare the release rate of the drug from the various nanoemulsion formulations

(Telmisartan gel TG, NE5 and NEG) all having the same quantity of Telmisartan (20 mg). The release rate of NE5 (86.22%) was found to be more as compared to NEG (72.35%) and TG (12.31%) as shown in fig. 9. Here it was observed that the release of NE5 is slightly more than NEG because the gel formulation provides a higher diffusional resistance for drug release. The comparison between NE5, NEG and TG showed that even though the release rate of NEG was less than NE5, it was significantly more than that of TG, due to presence of the drug nanocarriers.

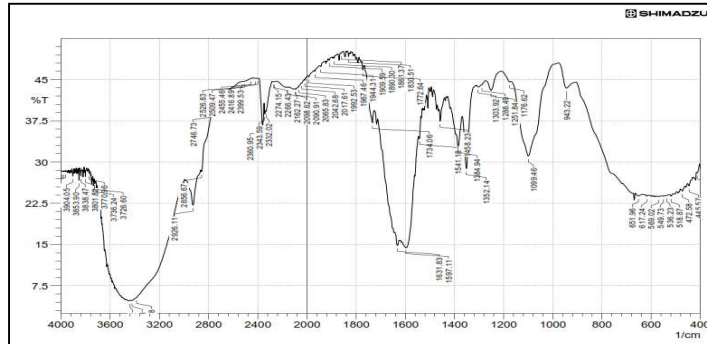


Fig. 7: FTIR Spectrum of Telmisartan nanoemulsion

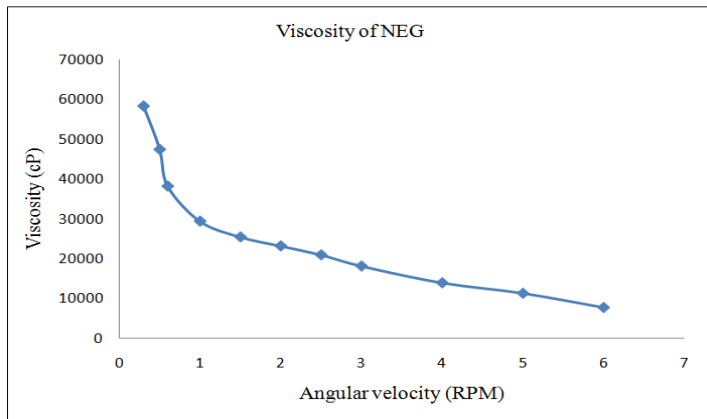


Fig. 8 Viscosity of NEG-2 at 25°C

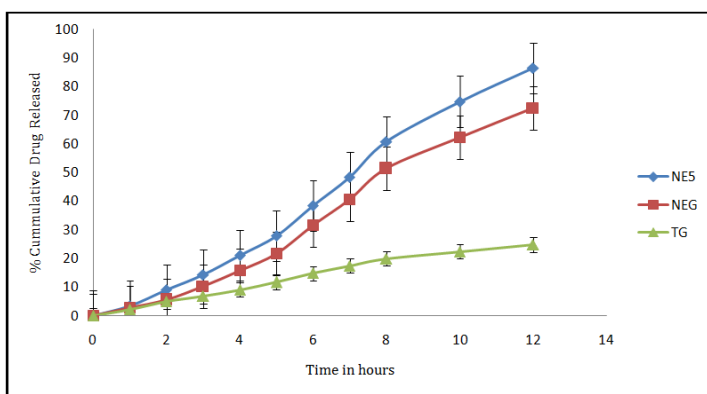


Fig. 9: In-vitro drug release of Telmisartan through excised rat skin

Table: 6 Permeation analysis data

Formulation code	Jss±SD* (µg/cm²/h)	Kp±SD* (cm/h)*10-2	Er*
TG	118.84±6.83	0.594±0.28	-
NE5	1393.2±7.14	6.97±0.21	11.73
NEG	1154.3±5.35	5.77±0.27	9.71

\*The values represented as mean±SD (n=3)

### Permeation data analysis

Permeability parameters like a steady-state flux ( $J_{ss}$ ), permeability coefficient ( $K_p$ ), and enhancement ratio ( $E_r$ ), were significantly increased in nanoemulsions NE5 and the NEG formulation as compared to telmisartan gel (TG). The flux value was found to be  $(118.8 \pm 6.83 \mu\text{g}/\text{cm}^2/\text{h})$  of normal gel (TG) in comparison to NE5  $(1393.2 \pm 7.14 \mu\text{g}/\text{cm}^2/\text{h})$  and NEG  $(1154.3 \pm 5.35 \mu\text{g}/\text{cm}^2/\text{h})$ . Permeability coefficient ( $K_p$ ) and Enhancement ratio ( $E_r$ ) of NE5 and NEG are described in table 6.

This result indicates higher permeability of drug through the skin because of the presence of nanocarriers in the formulations. The flux of NE5 is more than NEG means gel formulation provides prolonged

drug release behavior as compare to nanoemulsion. Moreover, it can be thought that NE5 and NEG excipients contain permeation enhancers like Acrysol® EL 135 and Carbitol®, which were also responsible for the increased permeation ability in comparison to the normal gel. Faster release of the drug from nanoemulsion and nano emulsion gel could be due to small size of the droplet which permits faster release of the drug.

### In vivo studies

Telmisartan content in the rat blood was determined using a validated HPLC technique, the following are the pharmacokinetic parameters of the telmisartan loaded gel (TG) and telmisartan nano emulsion gel (NEG).

Table: 7 Pharmacokinetic parameters

Formulation code	t (max) (hours)	C (max) ( $\mu\text{g}/\text{ml}$ )	AUC <sub>0 to ∞</sub> ( $\mu\text{g}\cdot\text{h}/\text{ml}$ )
TG	8±0.09	5.7±0.09	221.08±20.86
NEG	2±0.11	6.2±0.11	344.37±23.82
	***p<0.0001	*p<0.05	*p<0.05

\*\*\*p – extremely statistically significant, \*p – statistically significant

From table 7, it can be observed that AUC<sub>0 to ∞</sub> was greater when telmisartan was administered as nanoemulsion gel compared to the conventional gel formulation (TG) and that the areas under the curves for the nanoemulsion gel and telmisartan gel were statistically significant ( $p < 0.01$ ). The peak plasma concentration for nanoemulsion gel was  $6.2 \mu\text{g}/\text{ml}$  which was higher when compared to telmisartan gel ( $5.7 \mu\text{g}/\text{ml}$ ). As noted in Table-7, the mean  $t_{max}$  associated with TG is 8 hours which is notably longer than NG (i. e. 2 hours). These results showed that it was possible to improve the bioavailability of poorly soluble drug like telmisartan if given as nanoemulsion gel. This could be because of high content of surfactants in the nano emulsion which increase permeability by disturbing the cell membrane. There could be improved lymphatic absorption due to long chain oils. There is an increase in the surface area of the drug in a nanoemulsion which enhances their solubility and permeation through tissue barriers.

### CONCLUSION

In this study, Telmisartan loaded nanoemulsion gel was formulated to improve bioavailability of the drug. The nanoemulsion gel NEG containing Labrafil M 2125 CS (14.3%) as oil, Acrysol EL135 (30.84%) as surfactant, Carbitol (15.42%) as co-surfactant and (32.44%) water; 20 mg drug and 1% w/w carbopol was optimized. The optimized formulation was compared to conventional gel formulation and it showed higher permeation rate *in vitro* and *in vivo* which justifies the nanoemulsion gel to be a promising carrier for transdermal delivery of Telmisartan.

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### CONFLICT OF INTERESTS

Declared None

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