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Original Article

Formulation development and evaluation of Telmisartan Nanoemulsion

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ABSTRACT: The aim of the present study was to development of oral nanoemulsion formulation of telmisartan belong to BCS class 2nd, It have low solubility and low permeability. Therefore, oral nanoemulsion containing Telmisartan was prepared to increase its solubility and bioavailability rate. The o/w nanoemulsion was prepared by screening the excipients from the nanoemulsion region of pseudo-ternary phase diagram. Oleic acid optimized as an oil phase based on higher solubility study. Surfactant (Tween 80) and co-surfactant (PEG 200) were mixed (Smix) in different volume ratios (1:1, 1:2, 1:3, 1:4, 2:1, 3:1..). The optimized formulation containing Oleic acid (13.6%), Tween 80 (23.9%), PEG 200 (7.9%) and Methanol (54.6%) as oil, surfactant, co-surfactant and aqueous phase in Smix was prepared. Formulated nanoemulsions has to be evaluated for UV, FTIR, in vitro drug release, viscosity, particle size, product stability at accelerated conditions compared to the conventional formulation. Optimized oral nanoemulsion showed increase bioavailability.

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INTRODUCTION

The new molecular entities (NMEs) displays low solubility in water leading to poor bioavailability. Therefore, suitable formulations play an important role to improve the solubility and bioavailability of such drugs. Various options available to overcome the hurdle include solid dispersions, salt formation, micronisation, use of microspheres, co-grinding, lipid based formulations and many others. Lipid based formulation attracted wide attention in enhancing drug solubilization in the gastrointestinal tract (GIT) and to improve the oral bioavailability [1-5]. Nano-emulsion are Nano-sized emulsions, provides improvement the delivery of active pharmaceutical ingredients. They are thermodynamically stable isotropic system where two immiscible liquids are mixed form a single phase by means of an emulsifying agent. *i.e.* oil, surfactant and co-surfactant. Small size droplets decrease the interfacial tension *i.e.*the surface energy per unit area, between the oil and water those of the emulsion. It is defined as an oil in water (o/w) emulsion with mean droplet diameters ranging from 50 to 1000 nm usually the average droplet size in between 100 – 500 nm.

Telmisartan is an antihypertensive agent which selectively inhibits the type 1 angiotensin 2nd receptor. Telmisartan undergo significant first pass metabolism, hence low bioavailability. The objectives of the present study were to develop potential nanoemulsion formulations for oral delivery of telmisartan.

Advantages of nanoemulsion:

1. Increase the rate of absorption.
2. Helps solubilize lipophilic drug
3. Increases bioavailability
4. Various routes like topical, oral and intravenous can be used to deliver the product.
5. They do not show the problems of inherent creaming,flocculation,coalescenceandsedimentation.
6. Nanoemulsions have higher surface area and free energythat make them an effective transport system.
7. Less amount of energy requirement.
8. It is thermodynamically stable.

Types of Nanoemulsion: Depending on the composition there are three types of nanoemulsions:

1. Oil in water nanoemulsions wherein oil droplets are dispersed in the continuous aqueous phase.

2. Water in oil nanoemulsions wherein water droplets are dispersed in the continuous oilphase.

3. Bi-continuous nanoemulsions wherein micro domains of oil and water are inter dispersed within the system.

Components of nanoemulsion: Nanoemulsions contain main components:

1. **Drug:** Poorly soluble drug *e.g.* CBS, Diclofenac, Ramipril.
2. **Oil phase:** Oleic acid, Olive oil, Castor oil.
3. **Aqueous phase:** Methanol, ethanol.
4. **Surfactant:** Tween 80, Tween 20, Span 20.
5. **Co-surfactant:** PEG 200, PEG 400, Polysorbate 80.

Methods of preparation of nanoemulsion:

Factors to be considered during preparation of nano-emulsion:

a. Surfactants must be carefully chosen so that an ultra-low interfacial tension (< 10-3 mN/m) can be attained at the oil / water interface which is a prime requirement to produce nanoemulsions.
b. Concentration of surfactant must be high enough to provide the number of surfactant molecules needed to stabilize the micro droplets to be produced by an ultra-low interfacial tension.
c. The interface must be flexible or fluid enough to promote the formation of nanoemulsions.

Phase Inversion Method: Fine dispersion is obtained by chemical energy resulting of phase transitions occurthrough emulsification method. The phase inversion temperature (PIT) method was introduced based on the principle of changes of solubility of polyoxyethylene type surfactant with temperature.

Sonication Method: In this method, the droplet size of conventional emulsion is reduced with the help ofsonication mechanism. Only small batches of nanoemulsion can be prepared by this method.

High Pressure Homogenizer: This method is performed by applying a high pressure over the system havingoil phase, aqueous phase and surfactant or co-surfactant. The pressure is applied with the help of homogenizer.

Microfluidizatiion: Microfluidization technology makesuse of a device called 'MICRO FLUIDIZER". Thisdevice uses a high pressure positive displacement pump (500-200 PSI) which forces the product through the interaction chamber, consisting of small channels called micro channels. The course emulsion is into a micro fluidizer where it is further processed to obtain a stable nanoemulsion.

MATERIAL AND METHOD

Material: Telmisartan was obtained from yarrow chem product (Mumbai India).Oleic acid, olive oil, isopropyl myristate, PEG 200, PEG 400 gift sample from CDH New Delhi. Tween 80, Tween 20 were from himediya Lab Mumbai. All other chemicals used in the study were of analytical grade.

Methods:

Screening of oil: The oil was selected on the basis of their solubilizing capacity of telmisartan. The solubility of telmisartan in various oils was determined by adding an excess amount of drug in 5 mL of selected oils Oleic acid, IPM, ethyloleate, and Castor oil) in 15 mL capacity stoppered vials and kept under magnetic stirring at temperature of 25 ± 1.0˚C for 72 hours. The equilibrated samples were centrifuged at 3000 rpm for 15 min to separate the undissolved drug. The supernatant was taken and filtered through 0.45 µm membrane filter. 0.25 ml of the filtrate was diluted 1000 times with methanol and the absorbance of the sample was determined using UV-Visible spectrophotometer (UV-1800, Shimadzu) at 250 nm. The concentration of drug was determined from the regression equation obtained by plotting the standard curve of absorbance *versus* concentration of telmisartan in methanol (µg/ml).

Screening of surfactants: Surfactant was selected on the basis of its emulsification ability of oil in water. The modified method as reported earlier was used for the screening. The accurate amount (300 mg) of surfactant was added in to 300 mg of the selected oily phase and the mixture was gently homogenized at 45–60˚C. The isotropic mixture of 50 mg was accurately weighed and diluted with distilled water to yield a final emulsion volume of 50 ml. The ease of formation of emulsion was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The emulsion was allowed to stand for 2 hrs to note for any change in turbidity through visual observation and their transmittance was assessed at 250 nm bycolorimeter (6051 Jenway, UK) using distilled water as blank.

Screening of co-surfactants: The co- surfactant was selected by mixing 100 mg of co-surfactant with 200 mg of the previously selected surfactant and the surfactant-co-surfactant (S_{mix}) was added to the selected oil phase. The mixture was gently heated at 45– 60˚C for homogenizing the components. The 50 mg of isotropic mixture was accurately weighed and diluted to 50 ml with double distilled water to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 hr to note for any change in turbidity and their transmittance was assessed at 250 nm by colorimeter using distilled water as blank. As the ratio of co-surfactants to surfactant/s is the same, the turbidity of resulting nanoemulsions will help in assessing the relative efficacy of the co-surfactants to improve the nanoemulsification ability of the surfactants.

Pseudo-ternary phase diagram: The pseudo-ternary phase diagrams were developed using the aqueous titration method.

Surfactant (Tween 80) and co-surfactant (PEG 200) were mixed(S_{mix}) in different volume ratios (1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1).

Oleic acid optimized as an oil phase based on the solubility study. For each phase diagram, oil (olive oil) and specific S_{mix} ratios were mixed thoroughly in different volume ratios from 1:7 to 7:1. Different combinations of oil and S_{mix} (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1) were made for the study to delineate the boundaries of the phases precisely formed in the phase diagrams [8,9,10].

Aqueous phase was slowly titrated for each combination of oil and S_{mix} separately. 5ml of aqueous phase was added at each interval up to 50ml under magnetic stirring and visually observed for phase clarity and flowabilty. Calculations for other ratios of oil and S_{mix} were also done. The physical state was plotted on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second representing the oil phase, and third representing a mixture of surfactant and co-surfactant (S_{mix}) at a fixed volume ratio [11, 13, 16, 18].

Figure 1: visual observations of transparent and easily flowable o/w nanoemulsion made by different oil and S_{mix} ratio

Selection of formulations: Based on the NE region of each phase diagram different formulations are selected in which drug is incorporated into the oil in following basis:

1. 30 mg of Telmisartan was selected as the dose for incorporation into the oil phase.
2. 2 mL was selected as the NE formulation for convenience.
3. The oil should be in such a concentration that it solubilizes the drug (single dose) completely. 10 mg of Telmisartan will dissolve easily in 0.2 mL of oil (10% of 2 mL).
4. Different concentration of oils was selected from each phase diagram, at a difference of (5% 10%, 15%, 20%, 25%, etc) from the NE region.
5. For each 5 % of oil selected, the formula that used the minimum concentration of S_{mix} for its NE formulation was selected from the phase diagram.

Preparation of nanoemulsion by Bath Sonicator: Drug loaded nanoemulsion formulations were prepared using a Digital ultra-sonication method. Separately, in the oil phase, consisting of 5 ml of oleic acid the drug was added to the oil phase and stirred with the help of magnetic stirrer. The surfactant and co-surfactant mixture was prepared by S_{mix} ratio (1:1, 1:2, 1:3, 2:1, etc.) Gradually, the S_{mix} (2:1) was added to the oil phase under stirring conditions (Table 1). The oil droplet particle size in the course emulsion formed was further reduced by Digital ultra-sonication at 21% amplitude and 50% duty cycle using Digital ultra-sonicator (Sonic – vibra cell Bandelin RK 100 H, Germany) ultrasound instrument for 15 minutes [6,7].

Characterization of nanoemulsion Characterization of nanoemulsion:

Phase behaviour study: This study is a characterization and optimization of ingredients (surfactant, oil phaseand aqueous phase). Generally, the study is necessary in case of nanoemulsion formulation prepared by phase inversion temperature method and self-emulsification method in order to determine the phase of nanoemulsion and dispersibility. Study is done by placing the different ingredients of nanoemulsion by varying the concentration in glass ampoules and thoroughly homogenized at a certain temperature for a time until equilibrium anisotropic phase can be identified by polarized light.

Table 1: Composition of nanoemulsion excipients

Sr. no.	Formulations	Drug (telmisartan) in mg	Oleic acid in ml	Tween 80 in ml	PEG 200 in ml	Methanol
1	NT1	30	5	5	2.6	2.5
2	NT2	30	5	5	2.6	2.5
3	NT3	30	4	3.8	3.8	3.6
4	NT4	30	4	3.8	3.8	3.6
5	NT5	30	3	3	4.6	4.5
6	NT6	30	3	3	4.6	4.5

Particle size analysis: Generally, in case of nanoemulsion dynamic light scattering (DLS) method is used forthe measurement of particle size and their distribution.

Surface Charge Measurement: The surface zeta potential of nanoemulsion is predicted with the help of minielectrode.

Transmission Electron Microscopy: This method is used to observe the morphology of nanoemulsion.

Viscosity: Viscosity will be measured to ensure the better delivery of the formulation.

Stability of Nanoemulsions: Stability studies are performed on nanoemulsions by storing them at refrigeratorand room temperatures over a number of months. The viscosity, refractive index and droplet size are determined during this period of storage. Insignificant changes in these parameters indicate formulation stability.

Accelerated stability studies can also be performed. In this case, nanoemulsion formulation is kept at accelerated temperatures and samples are withdrawn at regular intervals and analyzed for drug content by HPLC. The amount of drug degraded and remaining in nanoemulsion formulation is determined at each time interval.

Polydispersity Index: The average diameters and polydispersity index of samples are measured by photon correlation spectroscopy. The measurements are performed at 25^{o}C using a He-Ne laser.

pH: The apparent pH of the formulation is measured by pH meter.

Drug Content: Drug content is determined by reverse phase HPLC method Using C18 column.

Zeta Potential: Zeta potential is a technique which is used to measure the surface charge properties and further the long term physical stability of nanoemulsions, the instrument which is used to measure the surface charge is known as Zeta PALS

Preparation of Standard Curve of Telmisartan in Methanol:

Preparation of Stock Solution: Accurately weighted Telmisartan (10 mg) and transferred in a 100 ml volumetric flask and dissolved in a small amount of methanol by shaking gently and volume was made up to 100 ml with methanol. A working standard stock solution (1o µg/ml) was obtained by diluting 1o ml of this solution to 10 ml by methanol in a volumetric flask. 1 ml, 2 ml, 3 ml …. 9 ml, of stock solution were transferred quantitatively into series of 10 ml volumetric flask and volume was made up to 10 ml to produce solutions of concentration ranging 1 to 10 µg/ml.

Determination of λ_{max} of Telmisartan:

The λ max of drug sample was determined by scanning 10 µg/ml standard stock solution in the range from 200-400 nm using Shimadzu – 1800 UV spectrometer. The scan is shown in figure 2.

Preparation of Calibration curve: The calibration curve of Telmisartan was prepared in methanol by preparing 1 to 10 µg/ml dilutions. Aliquot of 1,2,3,...10 ml of stock solution (10 µg/ml were transferred quantitively into series of 10 ml volumetric flask and volume was made up to 10 ml to produce solutions of concentration ranging 1 to 1o µg/ml. The absorbance of solution was determined at λ_{max} (296 nm) against blank (methanol).

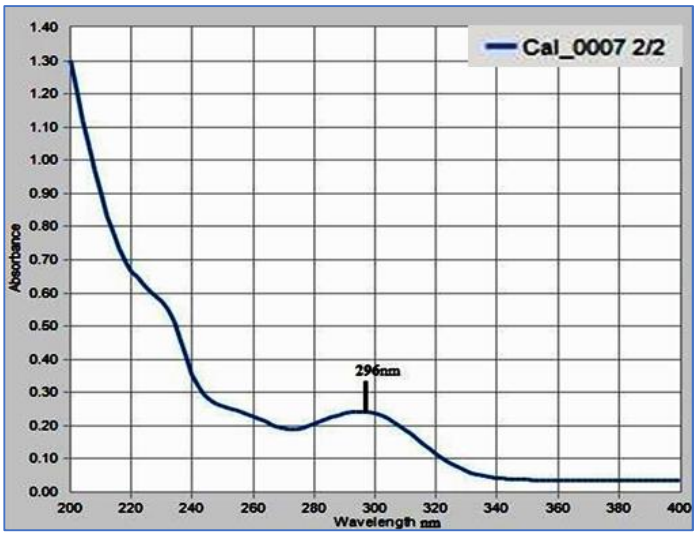

Figure 2: Spectra of telmisartan

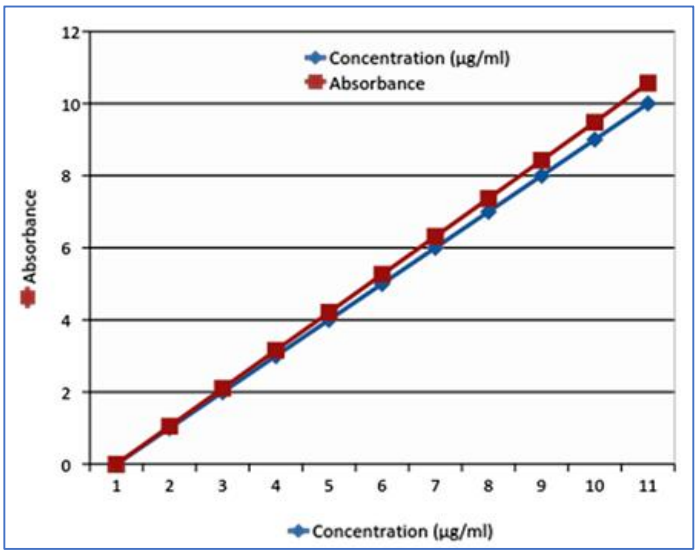

Figure 3: Standard curve of telmisartan in methanol

Table 2: Calibration curve value Telmisartan in Methanol at λ_{max} 296 nm

Concentration (µg/ml)	Absorbance
0	0
1	0.049
2	0.101
3	0.156
4	0.207
5	0.262
6	0.313
7	0.367
8	0.426
9	0.483
10	0.568

Identification of Drug by Infrared Spectroscopy:

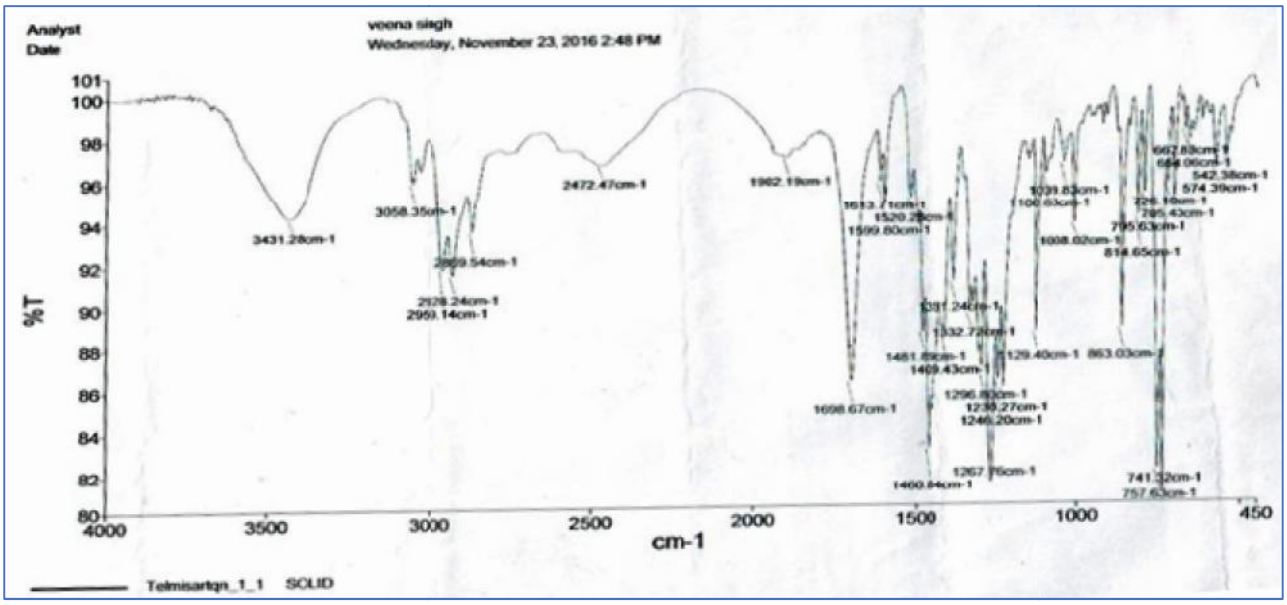

Figure 4: FTIR spectra of telmisartan

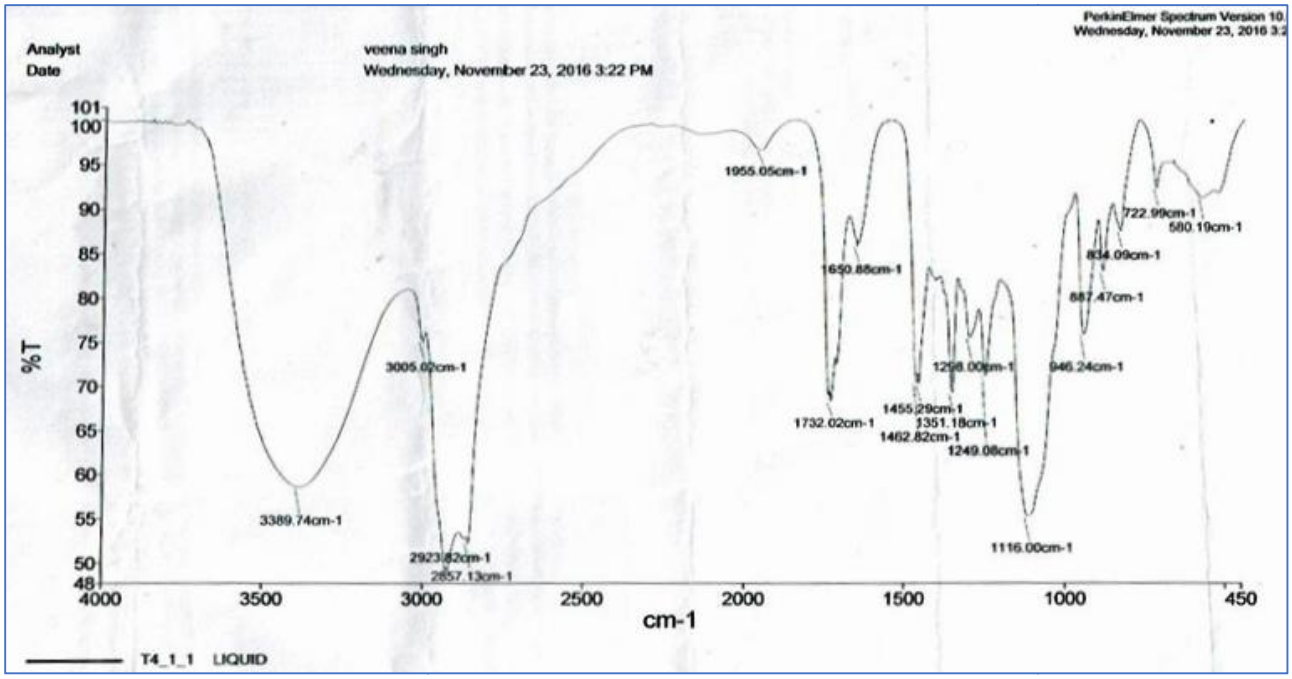

Figure 5: FTIR Spectra of drug polymer interaction

Table 3:Interpretation of FTIR Spectra of telmisartan

Functional Group	Observed Values for Telmisartan (cm^{-1})
Telmisartan: O-H Stretching	3421.28
C-H Stretching	2928.24
C-H Stretching	2869.54
C-H Stretching	2472.47
O-H Stretching	1962.19
C-C Stretching	1613.71
N-H Stretching	1599.80

Drug polymer interaction (T4):

Table 4: Interpretation of FTIR Spectra of drug-polymer interaction

Functional Group	Observed Values for Telmisartan (cm^{-1})
O-H Stretching	3389.74
C-H Stretching	2923.82
C-H Stretching	3005.02

O-H Stretching	1955.05
C-C Stretching	1650.88

In vitro* drug release:In vitro* drug release for the nanoemulsionformulation should be done in order to measure and detect the formulation that release the maximum amount drug (telmisartan) release from the nanoemulsion formulation. This test was performed in 500 mL of Phosphate buffer pH 7.4 using USP Dissolution apparatus Type II at 75 rpm and 37±0.5^{o}C. 2 mL of nanoemulsion formulation containing single dose 10 mg of Telmisartan was placed in a dialysis bag (Himedia dialysis membrane 150). Samples (5mL) were withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8 and 12 hrs) and an aliquot amount of phosphate buffer was replaced. The release of drug from nanoemulsion formulation was compared with the conventional tablet formulation and the suspension of pure drug. The samples were analysed for the drug content using UV-Visible spectrophotometer at 296 nm [14].

Table 5: Comparative *in vitro* release data for various formulations

Time (hours)	Cumulative percentage release (%)							
	NT1	NT2	NT3	NT4	NT5	NT6	Suspension	Telma (Tablet)
0	0	0	0	0	0	0	0	0
0.5	29.8±0.2	35.6±0.7	25.6±1.8	36.2±1.4	20.2± 1.5	26.4±1.6	15.2±1.3	14.3±1
1	42.3±0.8	57.43±1.16	35.67±1.4	57.88±1.5	31.0±1.08	37.62±1.2	19.1±1.06	17.2±1.07
1.5	58.2±0.4	63.9±1.2	43.9±1.7	64.18±1.32	39.4± 1.8	44.9±1.5	23.09±1.9	22.1±1.3
2	62.8±1.6	68.53±1.8	48.2±0.7	68.80±1.13	45.63±1.9	49.20±0.3	26.1±1.8	25.2±2.1
4	74.6±1.5	76.7±0.5	56.53±1.5	82.06±1.7	52.64±1.7	57.55±1.7	29.3±1.6	27.9±2.6
6	80.2±1.6	86.5±1.6	69.02±1.8	88.14±2.9	56.22±2.8	71.02±1.6	36.1±1.9	31.1±1.6
8	89.2±1.5	92.65±1.2	78.5±1.2	96.16±1.8	71.28±2.5	79.2±1.4	41.08±1.5	39.2±1.2
12	94.1±1.8	94.27±1.8	82.28±1.3	99.09±0.4	76.8±2.5	83.26±1.9	46.8 ±2.9	44.4±0.2

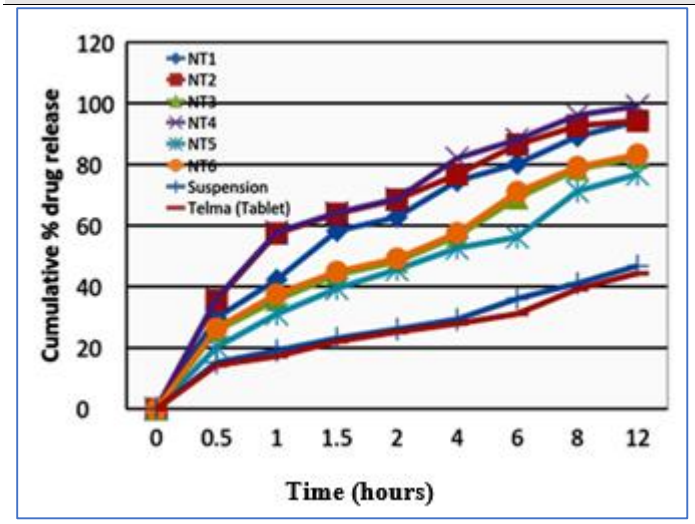

Figure 6: *in vitro* release profile of telmisartan formulation in 7.4 pH buffer

Thermodynamic stability tests: Selected formulations were subjected to different thermodynamic stability tests.

Heating cooling cycle: Between refrigerator temperature 4°C and 45°C of 6 cycles with storage at each temperature of not less than 48 h were conducted. Formulations, which were stable at these temperatures, were subjected to centrifugation.

Centrifugation:

Those formulations that passed were centrifused at 3500 rpm for about 30min by using centrifuge. The formulations that did not phase separated were taken to the further tests.

Freeze thaw cycle: Between – 21^{0}C and +25^{0}C three freeze thaw cycles with storage at each temperature for not less than 48 h was done for the formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility tests

Droplet size and zeta potential measurement: Droplet size and zeta potential of the nanoemulsion were determined by photon correlation spectroscopy that analyses the fluctuation in light scattering due to Brownian motion of the particles using a Zetasizer (1000 Malvern Instruments UK). The formulation (0.1 ml) were dispersed in 50 ml of DI water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering were monitored at 25°C at 90° angle.

Table 6: Droplet size, Zeta potential and polydispersity determination of NE Formulation

Sr. no.	Formulation code	Partical size(nm)	Polydispersity Index (PDI)	Zeta potential (mV)
1.	NT 1	107.5	1.00	-43.0
2.	NT 2	48.26	1.00	-1.27
3.	NT 3	86.43	0.98.2	-42.28
4.	NT 4	36.49	0.940	-47.1
5.	NT 5	105.56	0.982	-41.58
6.	NT 6	89.60	1.00	-38.65

Viscosity, Refractive index and percent transmittance: Viscosity of the formulations (0.5 g) was determined as such without dilution using Brookfield DV-II ultra-viscometer (Brookfield Engineering Laboratories,Inc., Middleboro, MA) using spindle # CPE 40 at 25 ± 0.5ºC. The software used for the calculations was Rheocalc V2.6. The refractive index of the system was measured by an Abbe refractometer (Bausch and Lomb optical company, NY) by placing 1 drop of nanoemulsion on the slide. The percent transmittance of the system was measured at 650 nm using UV spectrophotometer (Shimadzu, Japan) keeping distilled water as blank [6].

Transmission electron microscopy:TEM analysis should be done for the formulated nanoemulsion to determine the globule size of oil present in the nanoemulsion. This can be operating at 200 kV capable of point to point resolution. To perform the TEM observations, the nanoemulsion formulation was diluted with water (1/100). A drop of the diluted nanoemulsion was then directly deposited on the holey film grid and observed after drying.

Stability studies of optimized formulation: Stability studies on optimized nanoemulsion were performed by keeping the sample at 4±0.5°C, 25±0.5°C and 40±0.5°C. These studies were performed for the period of 3 months. The droplet size, viscosity, refractive index and conductivity were determined at 0, 1, 2 and 3 months [17].

RESULT AND DISCUSSION

Solubility of Telmisartan: The solubility of telmisartan in various oils, surfactants and co-surfactants is presented in Table 7. The solubilizing efficiency of the oily phase for the drug is the key determining factor for oil selection. Amongst the various tested oils, oleic acid had the largest solubilizing capacity for telmisartan (82.01±1.77mg/ml), so it was chosen for nanoemulsion formulation. The solubility of telmisartan in tween 80 (51.50±1.2 mg/ml) was higher than in Tween 20 (40.07±2.05 mg/ml). In addition, telmisartan exhibited the highest solubility in tween 80 among the tested surfactants (51.50±1.2 mg/ml). Hence, a Tween 80 was chosen as surfactant "S_{mix}" for nanoemulsion formulation. Regarding co-surfactant selection, the solubility of the drug will be the perspective criteria particularly due to the substantially high dose of telmisartan. PEG 200 showed the maximum solubility of telmisartan (44.92±2.35 mg/ml) and so it was the co-surfactant of choice in the present study. Oleic acid, Tween 80 and PEG 200 are known to have inhibitory effect on P-glycoprotein efflux that is responsible for the low bioavailability of many drugs.

Table 7. Solubility of drug in different Oils, surfactant and co-surfactant

Sr. no.	Name of oils	Solubility (mg/ml)	Name of surfactant and co-surfactant	Solubility (mg/ml)
1.	Oleic acid	82.01±1.77	Tween 80	51.50±1 .2
2.	Isopropylmyristate(IPM)	17.22±0.25	Tween 20	40.07±2.05
3.	Olive oil	08.25±0.45	Labrasol	30.57±0.87
4.	Triacetin	02.81±1.79	Span 20	18.07±2.78
5.	Jojoba oil	07.57±0.97	Labrasol+Tween 80	48.23±2.73
6.	Castor oil	08.35±0.84	PEG-200	44.92±2.35
7.	Groundnut oil	05.25±1.52	PEG-400	38.92±2.35
8.	Triacetin+Oleic acid(1:1)	78.19±1,78	Carbitol	35.44±1.7

Thermodynamic stability tests: Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nanoemulsions from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for dispersibility test.

Table 8: Thermodynamic stability test of different selected NT formulation

Sr. no.	Formulation code	Freeze thaw cycle		Centrifugation	Heat & cooling cycle		Dispersibility test	Inference
		4 °C	45 °C		-21 °C	+25 °C		
1	NT 1	√	√	√	√	√	A	Pass
2	NT 2	√	√	√	√	√	A	Pass
3	NT 3	√	√	√	√	√	A	Pass
4	NT 4	√	√	√	√	√	A	Pass
5	NT 5	√	√	√	√	√	A	Pass
6	NT 6	√	√	√	×	√	B	Pass

Transmission Electron microscopy:TEM analysis for the formulation has to be done by using zetasizer. A "positive" image is seen using TEM.Some droplet sizes were measured using TEM, as it is capable of point to point resolution.

The droplets in the nanoemulsion appear 45.75 nm, 42.59 nm,42.55 nm dark and the surroundings are bright.

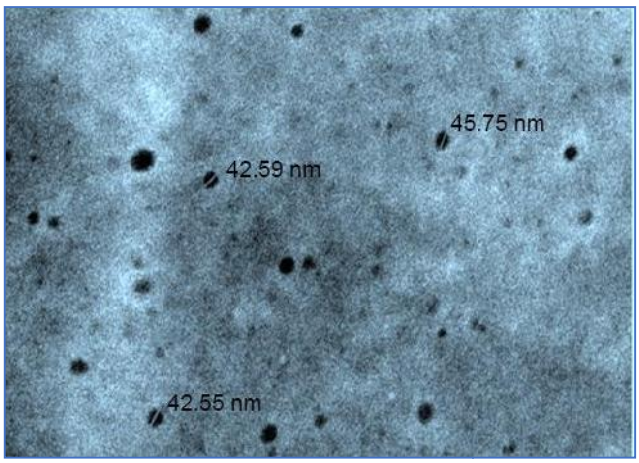

Figure 7: Transmission electron microscopy image

Droplet size, zeta potential and polydispersity measurement: Droplet size and zeta potential of the nanoemulsion were determined by photon correlation spectroscopy that analyses the fluctuation in light scattering due to Brownian motion of the particles using a Zetasizer (1000 Malvern Instruments UK). The formulation (0.1 ml) were dispersed in 50 ml of DI water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering were monitored at 25°C at 90° angle.

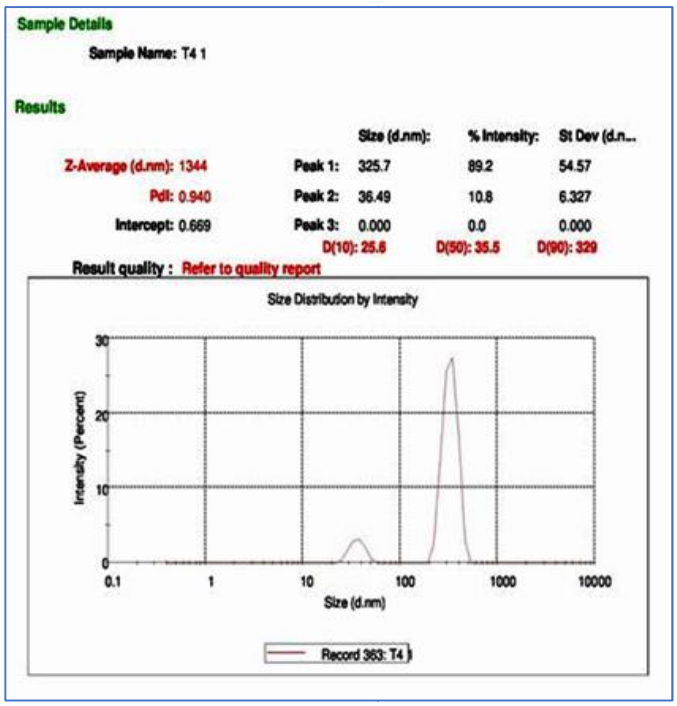

Figure 6: Droplet size and polydispersity determination by zetasizer

Table 9: Droplet size, Zeta potential and polydispersity determination of NT Formulation

Sr. no.	Formulation code	Particle size(nm)	Polydispersity Index (PDI)	Zeta potential (mV)
1.	NT 1	107.5	1.00	-43.0
2.	NT 2	48.26	1.00	-1.27
3.	NT 3	86.43	0.98.2	-42.28
4.	NT 4	36.49	0.940	-47.1
5.	NT 5	105.56	0.982	-41.58
6.	NT 6	89.60	1.00	-38.65

***In vitro* Release studies:**The highest release i.e. 99.09±0.4 % was obtained in NT4 can be detected 12 hours by performing Dissolution studies from 5 different nanoemulsion formulations (NT1 to NT6), and simple drug suspension, having same quantity 10 mg of Telmisartan and the amount of drug release is compared (Table 5).

Stability studies: All nanoemulsion formulation was characterized for droplet size, viscosity, pH, and RI for the period of three months. It was found that the droplet size, viscosity and RI of NT-4 formulation were not significantly changed during 3 months of storage period at 4^{o}C (Table 10 and Figure 8). These results indicated that the optimized formulation was stable as there were no significant changes in physical parameters.

Table 10: Stability studies of optimized nanoemulsion (NT4) were performed by keeping the sample at refrigerator temperature (4^{0}C), room temperature (25^{0}C) for the period of 3 months

Time (months)	Temperature (^{0}C)	Partical size	RI± SD
0	4.0±0.5	36.49	1.34
1	4.0±0.5	36.49	1.34
2	4.0±0.5	36.49	1.34
3	4.0±0.5	38.84	1.35
0	25.0±0.5	36.49	1.34
1	25.0±0.5	38.84	1.37
2	25.0±0.5	42.48	1.38
3	25.0±0.5	42.48	1.38

CONCLUSION

Telmisartan was successfully formulated as nanoemulsion formulation (NT4=99.09±0.4). Its exhibited faster and increase

bioavailability of telmisartan than marketed tablet regardless(44.4±0.2) of the type and pH of the dissolution medium. This improve the oral bioavailability of telmisartan formulation. The plan of work consists of compatibility test, contruction of the pseudo ternary phase diagram to know the range of nanoemulsion, selection of the formulation and incorporated of the drug, evaluation of the formulation and stability study.

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Conflict of interests:

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