Development, Characterization, and In Vitro Evaluation of Telmisartan Nanoemulsion

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Abstract

The Paper demonstrate the work been done for development of Nanoemulsion formulation of telmisartan belongs to BCS class II, which are known to have low solubility and low permeability. An Capmul MCM based Nanoemulsion formulation with Tween 80 as surfactant and PEG 400 as cosurfactant was developed for oral delivery of Telmisartan. A single isotropic region, which was considered to be a bicontinuous Nanoemulsion, was found in the pseudoternary phase diagrams developed at various Tween80: PEG 400: Capmu MCM ratios. The Nanoemulsion system was also investigated in terms of other characteristics, such as viscosity, pH, conductivity, clarity, particle size, in vitro drug release, product stability at accelerated conditions compared to the Conventional formulation. The developed Nanoemulsion system improved the solubility to get the desired invitro dissolution profile in release media to mimic the innovator product performance.

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Introduction:

The development of formulation for poorly water-soluble drugs has been challenging and it is the subject of much research, as approximately 40% of drugs are hydrophobic in nature. In such cases Nanoemulsion would be an ideal choice to crack the issues related with the solubility& Permeability. An emulsion is a system in which one fluid is dispersed in another with which it is immiscible. Macroscopic separation of the phases is prevented by the addition of a suitable surfactant. In the vast majority of emulsion research, one of the liquid phases is water. 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 molecules. A Nanoemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension. Because the droplet size is less than 25% of the wavelength of visible light, Nanoemulsions are transparent. The Nanoemulsion is formed readily and sometimes spontaneously, generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase. Telmisartan was selected as model drug due to its low aqueous solubility. Telmisartan is an orally active non-peptide angiotensin II antagonist that acts on the AT1 receptor subtype. Angiotensin II is the principal pressor agent of the renin-angiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation,
and renal reabsorption of sodium. Telmisartan works by blocking the vasoconstrictor and aldosterone secretory effects of angiotensin II

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![Chemical structure of Telmisartan](Image)

Figure 1: Chemical structure of Telmisartan

Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) are isotropic mixtures of oil, surfactant and/or cosurfactants, and a drug that spontaneously forms an oil-in-water Nanoemulsion upon gentle agitation with water. When dispersed in the gastrointestinal (GI) tract, the motility of stomach provides necessary agitation for emulsification. SNEDDS incorporated with a poorly water soluble drug demonstrates improved drug absorption since it maintains the drug in a solubilized state in the GIT tract(3). This study demonstrates development and characterization of liquid Nanoemulsion formulation using telmisartan as a model drug and encapsulated into softgelatin capsules.

**Materials and Methods**

Excipients evaluated for Development of Telmisartan SNEDDS formulation includes Tween 80 and Cremophor RH 40 as surfactants, Transcutol P, ethanol, labrafil, labrasol, propylene glycol and PEG 400 as cosurfactants and Labrafac FCC (a medium chain triglyceride), Capmul MCM, Captex, crodomol EO, olive oil, soya bean oil, capryol-90 as the oil. Self Nanoemulsifying (SNE) mixtures containing various proportions of these components were tested for their self-Nanoemulsification ability and were characterized by ternary phase diagrams. Based on these results, a particular mixture containing Tween 80-PEG 400-Camul MCM was selected and optimized for drug delivery purpose.

**Preliminary solubility evaluation for the screening of components:**

Cremophor EL was selected as surfactant due to its high solubilizing capacity and due to the acceptance nature of various routes, also it is listed in GRAS. The solubility of telmisartan was evaluated in several excipients which includes Capmul MCM, Captex, Arachis oil, Iso[ropyl myristate and Medium chain glyceride. Labrasol, transcutol, Polyethylene glycol 400, propylene glycol, Ethanol were tested as co-solvents. Solubility was determined by adding excess quantity of drug into the excipients and shake in Vibroshaker(Millipore, USA) for 3 hours. If the solution becomes clear added further quantit of drug to the excipients and continued shaking till 24 hours. Then the solution was filtered through 0.22 μm PVDF Filters, then the filtrate was analysed by HPLC.

Quantitative determination of telmisartan were performed by using Waters HPLC. 2695-module pump, waters 2465 dual λ, absorbance detectors. Waters empower Software was used for the data processing and peak analysis. Optimization of chromatographic conditions was carried out in two methods (1.isocratic; 2.gradient). In the case of isocratic method of analysis, various mobile phase compositions (acetonitrile and 0.1% acetic acid or pH 10 buffer) were tried with constant flow rate (1ml/min); Column C18 and Column temperature (25±10°C), with an initial system pressure of approximately 1500 psi. Sample (standard drug samples) was injected (volume of 25 μL) and the full spectrum was recorded from which data processing and peak analysis were performed by using water
empower software. These same conditions were also used for the determination of solubilizing capacity shown by formulations[2]

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Calibration curve of Telmisartan

The calibration curves were generated for different concentrations of telmisartan. The concentrations were chosen based on the sensitivity of the method for each compound. Accordingly, the concentrations of telmisartan from 5.0μg/ml to 50 μg/ml were chosen. Calibration curves were obtained by a linear regression of the peak area ratio of telmisartan shown in figure 4

Pseudoternary Phase Diagram Construction:

Based on solubility results, excipients were selected to perform NE region screening. Different amounts of Polysorbate 80 and each one of the selected co-surfactants and oil phases were mixed using vibro stirrer for 10 minutes. Then, water was added and samples were left to equilibrate using a thermal bath at 37°C (Varian, USA) for 1 hour. The adopted criteria used for considering a formulation as an NE was based on the visual analysis of the compositions searching for clear, single-phase, isotropic and low-viscous systems.

Preparation of Telmisartan-Loaded NEs.

Telmisartan-loaded NEs were prepared by weighing appropriate amounts of Surfactant, the co-surfactant, and oil phase selected according with previous adopted criteria; gentle magnetic stirring during 10 minutes at room temperature was applied so as to obtain homogenous samples, which were left to equilibrate using a thermal bath at 37°C (Varian, USA) for 1 hour. Next, three different amounts of Telmisartan were added and dissolved with magnetic stirring. Finally, the corresponding amount of water for each one of the selected compositions was added under agitation at room temperature.

Physicochemical Characterization of Telmisartan-Loaded NEs.

Viscosity was measured by using Brookfield Viscometer at 25°C. Formulation of pH was determined with a pH-meter (Mettler Toledo). Conductivity was assessed using an Accumet research AR20 at 25°C. Droplet size was analyzed with Mastersizer, Malvern Instruments. Samples were diluted to carry out the measurements and assays were performed at 25°C. The polydispersity index indicates the size distribution within a ME population. The zeta potential of the formulations was determined using the same equipment (Mastersizer, Malvern Instruments). Samples of the formulation were placed in the electrophoretic cell, where an electric field of about 25V/cm was applied. The NEs were first diluted in water (1:10), a sample drop was placed onto a grid covered with Formvar film and the excess was drawn off with a filter paper. Samples were subsequently stained with Brilliant Blue solution for 1 minute. Samples were finally dried in a closed container with silica gel and analyzed. The droplet diameter was estimated using a calibrated scale[3]. Chemical stability was performed using the HPLC equipment described for solubility assays (Waters HPLC), and the chromatographic conditions were also the same. For short time stability studies, samples were charged in vials in 40°C±2°C/75% RH and 5°C±3°C for a month, then samples were reanalyzed. Direct observation of the formulations was used to evaluate drug precipitation or other physical change during the evaluation period. The objective of thermodynamic stability is to evaluate
the phase separation and effect of temperature variation on NEs formulation. All the NEs prepared were centrifuged (Eppendorf Centrifuge) at 10,000 rpm for 10 min, and then they were observed visually for phase separation. Formulations that did not show any sign of phase separation after centrifugation were subjected to freeze thaw cycle. In a freeze thaw study, Telmisartan NEs were evaluated for two freeze thaw cycles between (−20°C and +25°C) with storage at each temperature for not less than 2 h[4].

Results and Discussion

Preliminary Solubility Evaluation.

Telmisartan resulted almost insoluble in olive oil, crodomol, soyabean oil, seasame oil and captex as well. Capmul MCM showed solubility near 10mg/ml (Figure 2). Therefore, only CapmulMCML were selected for the forthcoming screening. The selection of the oily phase is very important because the drug solubility in the formulation depends mainly on it[5,6]. So, this property results, fundamental in the search for high solubilizing capacity systems.

The release from the Nanoemulsion system can be tailored by the proper selection of the oil system. They also were significantly higher than Telmisartan solubility in water. Furthermore, the high solubility which stated that active compounds with an intermediate lipophilicity (Log P of 5.0 and above, being 7.9 the value of the Telmisartan) have a high tendency to be solubilized by phospholipids. Solubility of telmisartan in the seven co-surfactants and in PS 80 is depicted in Figure2. The highest solubilizing capacity was achieved with Tween 80 and polyethylene 400; therefore, both compounds were selected to act as coemulsifiers in the forthcoming NEs screening.

However, Telmisartan showed a considerable solubility in Transcutol and Labrasol, but it resulted significantly lower than the selected surfactants. Finally, all other cosurfactant was discarded because it was the cosurfactant with the lowest drug solubilizing capacity. Solubility of Telmisartan in Tween 80 was around 45mg/ml; however, it is expected that these results slightly impact on the final therapeutic agent solubilization. The most important factor that contributes to the final ME solubilizing capacity in poorly water soluble drugs is the solubility in the lipid internal phase[7,8].

There is a synergic effect regarding drug solubility in the NEs compared to the solubility in the isolated excipients. This means that, in some cases, the difference observed for solubilizing capacity is tenfold higher. Taking into account the composition of the NEs, the solubility seems to increase with the raise in the lipid phase content. Thus, the higher the surfactant percentage for the same lipid level, the higher the solubility in the NE.
Figure 2: Solubility of Telmisartan in oil phases (expressed in mg/ml).

Figure 3: Solubility of Telmisartan in surfactants (expressed in mg/ml).
Telmisartan Linearity

![Telmisartan Linearity](image)

**Figure 4: Telmisartan linearity**

Screening and Optimization of NEs.

Based on solubility results, the following excipients were selected to perform the preliminary Nanoemulsion screening: Tween 80 as surfactant and Polyethylene Glycol 400 as co-surfactants and Capmul MCM L as the oil phases. The selection included compositions with a relative proportion of Tween 80 lower than 30%, relative concentrations of each one of the oil phases between 10 and 20%; the level of the co-surfactants was fixed in 15%. None of these compositions containing transcutol and labrasol as cosurfactant, matched the adopted criterion for considering ME system and they were discarded for the next step of selection. In relation to Capmul MCM, promising results were observed in agreement with other authors; as it has been recorded medium chain monoglycerides are known for their ease of emulsification when compared to fixed oils or longchain fatty acids [9]. They also exhibit good solubilizing capacity. At this stage of the work, only NEs containing capmul mcm, polyethylene glycol, and Tween 80 were selected (10). For their pseudoternary phase diagrams construction, two different surfactant/co surfactant ratios: 0.8 and 0.2 were considered (Figures 5).
Physicochemical Characterization.

A significant lowering effect of pH values was observed due to the acidic nature of telmisartan was added. Conductivity values obtained for the selected compositions correspond to those of o/w NEs.

The low viscosity values are representative for NEs (Table 1). The differences observed for viscosity values might be the result of the interaction between NE droplets in oil/water systems. It is expected that tween 80 hydrophilic chains are strongly hydrated and connected with hydrogen bonds; this allows the interaction between the droplets, thus raising the viscosity values.

Since NE formation process is generally a random stirring process; the resulting delivery system may result in a polydisperse system in which different droplet sizes can coexist. This information is extremely valuable in practice because both stability and viscosity depend on the drop size distribution. The later in vivo or in vitro behavior depends on this property as well. The addition of Telmisartan did not significantly change droplet size of formulations comparing with empty ones, even at the highest Telmisartan concentration of 80mg/ml. This is an interesting advantage for the selected compositions, because the loading of a lipophilic active compound could result in an increase in the droplet size and, eventually, could compromise the system physical stability.

A short stability testing was carried out with selected formulations. For this purpose, Telmisartan 80mg/ml formulation were charged in vials in 40°C±2°C/75% RH and 5°C±3°C for a month and samples were reanalyzed for Assay and Related substances. Results demonstrated that all formulations
showed a 100 ± 2% of the initial content after a month of observation. Obtained values confirm the total solubilization of the drug and absence of rapid degradation. Regarding physicochemical values, no significant changes in the values measured at the beginning of the study were obtained after the studied period. No precipitation or change in appearance was observed by direct visual observation. None of the NE formulations has shown any sign of in-stabilization during the thermodynamic stability tests carried out.

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Table 1: Initial and Stability results compilation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
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<td></td>
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<tr>
<td>Related substances</td>
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<tr>
<td>Highest known Imp</td>
<td>BQL</td>
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<td>Sum of known</td>
<td>BQL</td>
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<tr>
<td>% Total imp</td>
<td>0.03</td>
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<tr>
<td>Physicochemical properties</td>
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<tr>
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<tr>
<td>Conductivity (uS/cm)</td>
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<tr>
<td>Droplet size (nm)</td>
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<td>pdI</td>
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</table>

BQL: Below Quantification Limit

In-vitro release behaviors

Release studies were performed using vertical passive diffusion cells (HTD 96,HT Dialysis, USA), with a cellulose membrane. The cellulose (molecular weight <12 000) membrane was first hydrated in the buffer solution at 20°C for 24 hours. The membrane was then clamped between the donor and receptor compartments of the cells. The receptor solution was 0.20 mL of phosphate buffer pH 7.4 containing 1% SLS (Sodium lauryl sulphate) to, and it was maintained at 37°C ± 0.5°C using a thermostatic shaker bath (Eppendrof, Germany) and was stirred at 200 rpm throughout the experiment. The donor compartment contained 0.2 ml of Nanoemulsion sample.

The aliquots (0.05 mL) withdrawn at specified intervals from receptor compartment were then replaced by a fresh receptor solution. The samples were immediately analyzed directly for drug concentration by using HPLC. Two replicates of each experiment were performed. Invitro dissolution study was performed for both conventional reference product tablet formulation and Telmisartan Nanoemulsion formulation and compared for the similarity factor and f2 values found to be more than 50%
Table 2: Invitro Dissolution characteristics

<table>
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<th>Time point</th>
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<th>Telmisartan Nanoeulsion (SGC)</th>
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<tr>
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<td>0</td>
</tr>
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<td>5</td>
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</tr>
<tr>
<td>45</td>
<td>102.3</td>
<td>109.4</td>
</tr>
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</table>

Figure 6: Comparative dissolution profile of Telmisartan soft gelatin capsule Vs Reference product (Telmisartan Tablets 80mg) in pH 7.5 Phosphate Buffer (Release media)
Conclusion

Self-Nanoemulsifying drug delivery system (SNEDDS) is known to improve dissolution characteristics of a poorly water soluble drug since they maintain the drug in a solubilized state in the GI tract. Using the optimized SNE mixture, Temisartan loaded Liquid SNEDDS were prepared, evaluated for their self-Nanoemulsification tendency and characterized. The resulting Nanoemulsions from the trial formulations showed a droplet size of approximately 200 nm and a neutral zeta potential.

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The present work describes a novel interdisciplinary rational screening for a NE composition, its optimization, and the corresponding in vitro performance evaluation in selected release media. The development included physicochemical properties evaluation and drug solubility in selected formulations. It found both physic chemical property and release profile found satisfactory to mimic the invivo performances. The experimental design began with the proposal of extensively studied excipients for the screening, after that, the first criterion adopted for excipient selection was based on solubilizing capacity and ability to form NEs shown by each one of the excipients. In this study, self-Nanoemulsifying (SNE) mixtures containing surfactant, cosurfactant and a medium chain triglyceride were prepared and their tendency to efficiently emulsify was evaluated. Upon aqueous dilution, such mixtures spontaneously emulsified forming an oil-in-water Nanoemulsion. This property was dependent on the composition of the excipients as well as their individual concentration in the mixture.

SNEDDS can be potentially used for delivering telmisatan and release from the Nanoemulsion formulation can be tailored based on the need to match our requirement by the proper selection of the excipient and composition.

References


