Preparation and Characterization of Linseed Oil Based Nanoemulsion for Transdermal Delivery of Losartan

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Abstract

Conventional delivery of losartan through oral route suffers from very low bioavailability (~38%). In the present work, we have explored the efficacy of nanoemulsion system for consisting of linseed oil, tween 80, span 80 (2:1) and water for transdermal delivery of losartan. While the average droplet size of the nanoemulsion lied in a range of 287 to 350 nm, zeta potential of -50mV was recorded. Exhaustive stability tests on the formulation containing the maximum entrapped drug (~78%) revealed that the nanoemulsion was stable both under conditions of stress, and in long term storage. The pH of formulation was recorded to be 5.38± 0.01 and was considered fit for transdermal applications. A low permeation flux through porcine skin of 1.39 μg/cm^2/h from the optimized formulation revealed that the nanoemulsion is an ideal carrier for delivery of losartan in lengthy therapeutic schedules.

Keywords: *nanoemulsion; linseed oil; transdermal; losartan*

INTRODUCTION:

The skin is the largest organ in the body and has a surface area of about 1.5 to 2 mm^2, hence the transdermal route providing most promising and favorable route and an area of attraction for the researchers [1]. The limitation of oral route can be successfully overcome by transdermal delivery. Besides more convenient and easy administration it also avoids the hepatic metabolism and gastrointestinal intolerance, and also immediate withdrawal is possible of the drug if necessary [2]. The limitations of transdermal drug delivery are principally associated with the barrier function of skin due to the present of stratum corneum as the drug molecules cannot readily pass through this impervious barrier [3]. Many works in the recent years have intended to design topical vehicle for controlled and on demand modification of drug permeation through the skin. Nanoemulsion plays a role as a vehicle for delivery [4]. The mechanism may involve in enhancing permeation through permeation enhancer which may present as ingredients and help reducing the diffusion barrier of stratum [5, 6]. However, such permeation enhancers and solvents are known to cause undesired effect on the skin upon prolonged use. Transdermal permeation can be improve by nano emulsion.

Nanoemulsion can be defined as o/w emulsions with mean droplet diameters ranging from 50-1000 nm, and are transparent thermodynamically stable and translucent dispersion and oil and water [7].Its contain a mixture of oil, surfactant, co-surfactant and an aqueous phase. Losartan is a model choice of drug for our study act as angiotensin II receptor antagonist widely used as an antihypertensive drug [8]. Oral administration of losartan is most common and traditionally use but in these route bioavailability is only 33% and significantly first pass metabolism occurs and the drug have very low molecular weight (461.01 Da) and also containing log P 4.5 with very short biological half-life (2h) [9,10]. Due to these properties it became more flexible for transdermal delivery. Biocompatible nanoemulsion improve the solubility and stability of the encapsulated drug [11].

Our main objective of the study is to develop and characterize linseed oil based nanoemulsion for transdermal delivery of losartan. A pseudo-ternary phase diagram was plotted in order to identify the self-emulsifying region, from which the most stable formulation was selected for further evaluations.

MATERIALS AND METHODS

Materials

Losartan potassium was obtained as a gift from the HOD, Department of Pharmaceutics, Dr. B.C. Roy College of Pharmacy & A.H.S., Durgapur (India). Tween 80 (sorbitan monolaurate) and Span 80 (sorbitan monooleate) were purchased from Loba Chemie (Mumbai, India). Linseed oil was obtained from Shiv Sales Corporation (New Delhi, India). All reagents used in the study were of analytical grade. Double distilled water obtained from laboratory purification systems was used for all experiments.

Construction of pseudo-ternary phase diagram (PTD)

The PTD was constructed using aqueous titration method as described by Malakar et al., 2014 [12]. The surfactant system (S_{mix}) was constituted by Span 80 and Tween 80 in a fixed proportion of 1:2. Linseed oil was the oil phase constituent, and aqueous phase was composed of freshly prepared double distilled water. Initially surfactants were mixed with oily phase in ratios ranging from 2:8 to 8:2 and was then titrated with water. After each titration, samples were subjected to high sheer homogenization (Bharat motors, India) at 8000 rpm for 10 min at room temperature. Each samples were inspected visually for clarity and phase homogeneity. Clear and transparent formulations identified as nanoemulsions were subsequently marked in the phase diagram using Microcal Origin 6.0 (Microcal Software, Inc., USA) to identify the nanoemulsion zone.

Preparation of nanoemulsion

Nanoemulsion formulations selected from the constructed phase diagram were prepared according to the composition illustrated in Table 1. 10 mg of the drug was dissolved in linseed oil for each selected formulation. S_{mix} was then added in an appropriate ratio and the mixture was equilibrated under magnetic stirring for 15 min. Water was subsequently added drop-wise with continuous stirring. The primary coarse emulsion thus formed, were further subjected to high sheer homogenization at a temperature under 20 °C.

Physicochemical characterization of nanoemulsion:

Mean globule size and polydispersity index (PDI) of the formulation was recorded using a Zetasizer Nano ZS series (Malvern Instruments, UK) equipped with a 4mW He-Ne laser (λ= 633 nm). Samples diluted 1000 folds were placed in the module and data were recorded in triplicates for each batch at 25 °C. Zeta potential analyses were performed by measuring the electrophoretic mobility using

the same instrument after dilution of the samples in 10 mL water.

pH values of the nanoemulsion were recorded at 25 ℃ by directly immersing the electrode of a calibrated pH meter into the undiluted formulations.

Drug entrapment efficacy

Losartan containing nanoemulsions were first exposed to centrifugation at 14,000 rpm for 20 mins in Remi cooling centrifuge (Remi, India) to separate the un-entrapped drug from the final formulation. 100 µL of the supernatant was diluted with methanol and the concentration of the drug was quantified through UV-Vis spectroscopy.

Stability studies

Different formulations were examined for their resistance to centrifugation stress. Aliquots of samples (10 mL) were subjected to centrifugation at 6,000 rpm for 20 min and observed for any evidence of creaming, cracking or phase separation.

Freeze-thaw stability of nanoemulsion was determined by exposing the formulation to three freeze-thaw cycles, which included freezing to –10 ℃ for 24 h in a freezer followed by thawing at 40 ℃ for 24 h. The formulation was then evaluated for particle size and zeta potential.

Shelf life stability study was conducted by storing the formulations at room temperature for 3 months. Various physical parameters such as clarity, phase separation, creaming, creaking, color, and odor were observed. Similarly, droplet size, zeta potential and pH were recorded. Only the formulations which complied the stability tests were considered for further studies.

Quantification of losartan

The quantification of losartan was performed using a Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu, Japan). An absorbance (y) vs. concentration (x) plot, $y=0.455x - 0.0134$, $R^2= 0.998$, was first generated and applied for losartan estimation throughout.

Preparation of skin

Goat ear skin for permeation studies was obtained from a local slaughter house. Then the hair were removed using an animal hair clipper and subsequently, full thickness of the skin was harvested. The fatty layer adhering to the dermal side was removed by surgical scalpel. The skin were washed with 50 mM PBS (phosphate buffer saline, pH 7.4), and stored at -20 ℃ until further use.

Ex vivo permeation studies

Ex vivo skin permeation study of the selected formulation was carried out using a vertical Franz diffusion cell with an effective diffusional area of 1.64 cm^2 and receptor compartment capacity of 90 mL. The excised skin was fixed between the donor and receiver compartment with stratum corneum side facing the donor compartment. The receiver compartment was filled with PBS which was stirred with a magnetic rotor at 500 rpm, and the entire assembly was placed in an incubator to maintain a temperature of 37± 0.5 ℃. The skin was initially allowed to equilibrate for a period of 1 hr. 2 mL of nanoemulsion was then introduced into the donor compartment and sealed with aluminum foil to prevent evaporation of water. 5 mL of samples were withdrawn at regular intervals and replaced by the same amount of PBS. The samples were filtered and amount of drug permeated was quantified using a UV-Vis spectrophotometer, by measuring the absorbance at 254 nm wavelength.

Permeation flux

The amount of drug (Q) from the nanoemulsion permeated through excised skin was plotted against the function of time. The slope and intercept of the linear portion of plots were obtained through regression method. The

steady state was calculated from the slope divided by the effective diffusional area [12, 13]

$$J_{ss} = (dQ/dt)_{ss} . 1/A$$

Where J_{ss} is the steady-state permeation flux (µg cm^{-2} h^{-1}), A is the area of skin (cm^2), $(dQ/dt)_{ss}$ is amount of drug passing through the skin per unit time at a steady state.

2.10 Statistical Analysis

All experiments were performed in triplicates using freshly processed sample and the results were reported as mean± standard deviations.

RESULT AND DISCUSSION

Pseudo ternary phase diagram (PTD)

Non-ionic surfactants such as Tween 80 and Span 80 were chosen for the formulation of linseed oil based nanoemulsions according to their HLB values and phase behaviour. The flexibility of surfactant film, its affinity for water, and the interfacial tension are important parameters involved in the formation of nanoemulsions. In this aspect, the construction

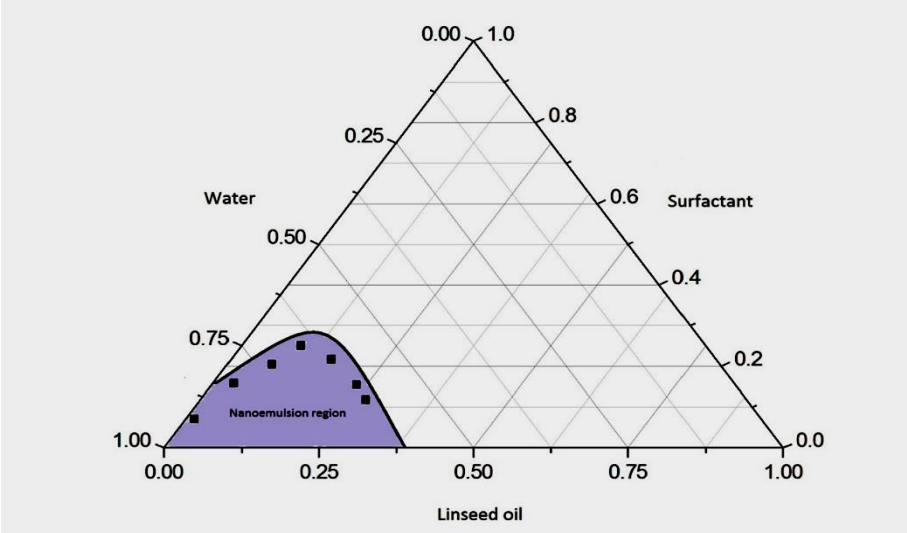

Figure 1: Pseudo-ternary phase diagram of linseed oil, surfactant (Span80 and Tween 80 in ratio of 1:2) and aqueous phase. The darkened region signifies the nanoemulsion zone.

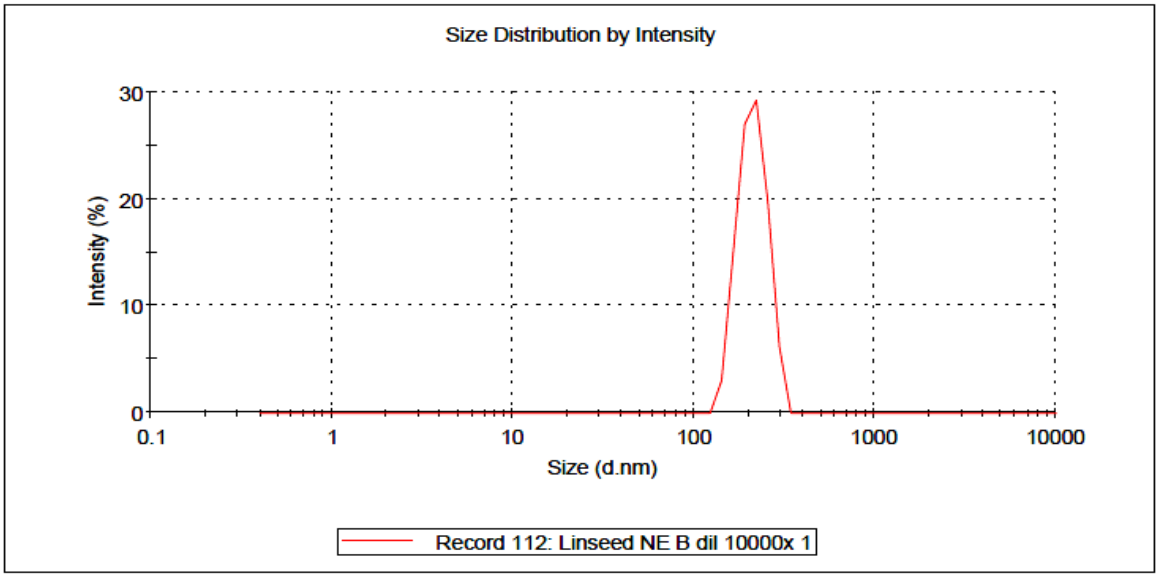

Figure 2: Droplet size distribution of losartan loaded nanoemulsion.

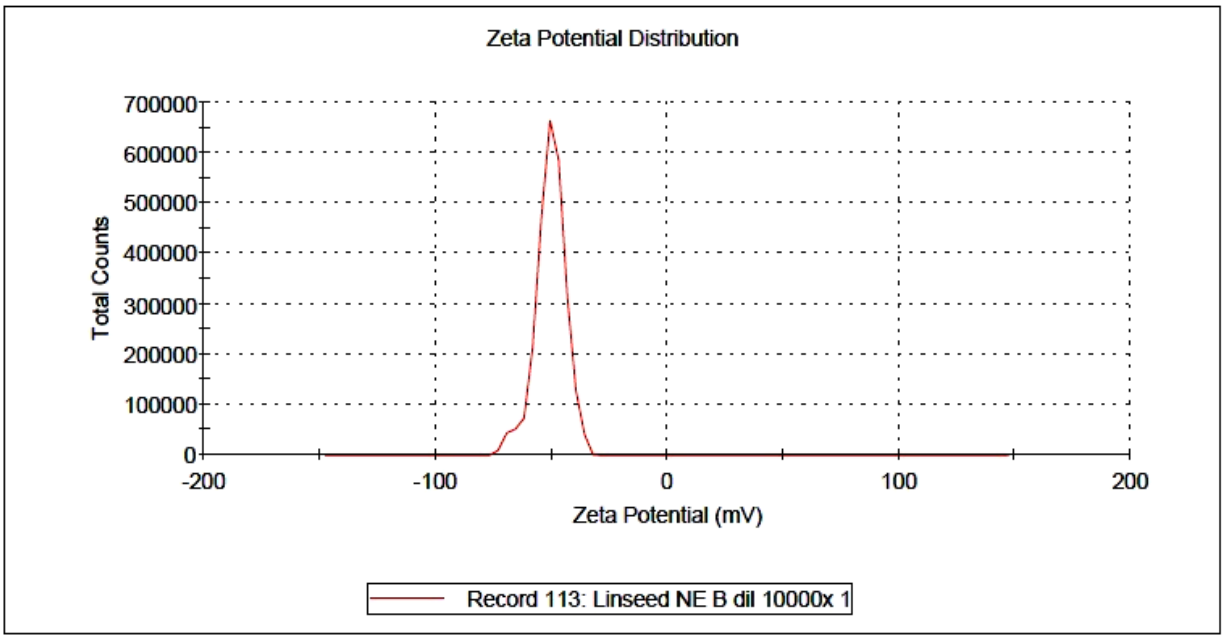

Figure 3: Zeta potential analysis of losartan loaded nanoemulsion (F5).

Table 1: Physico-chemical parameters for different nanoemulsion formulations

Codes	Average Droplet size(nm)	Polydispersity index	pH	Zeta potential (mV)	Drug entrapment (%)
F1	287.38± 3.4	0.35	4.60± 0.4	-38	72±0.7
F2	298.41± 3.61	0.41	4.64± 0.12	-40	73± 0.72
F3	297.42± 3.49	0.38	5.20± 0.38	-42	74± 0.8
F4	299.01± 3.7	0.34	4.81± 0.12	-51	74± 0.79
F5	300.02± 3.75	0.37	5.38± 0.01	-50	78± 0.87
F6	320.34± 3.82	0.39	5.05± 0.24	-48	75± 0.79
F7	350.73± 3.91	0.42	6.2± 0.32	-47	76± 0.81

*Results are expressed as mean± S.D.

of PTD has been widely employed to understand the balance among mixtures of surfactants, oil and water in the emulsion systems. In our work, PTD was constructed based on macroscopic observations of different type of dispersion obtained from mixtures produced. We used the aqueous titration method at room temperature (25±2 °C). Several sorts of dispersions, including conventional emulsions and nanoemulsions could be observed with the mixture of surfactants used. We have observed that the formation of a single-phase region at very low oil concentrations and high surfactants concentrations. The formation of a milky single-phase system in most part of the diagram suggests that the mixture of surfactants was able to minimize the surface tension between aqueous and oily phase, thus promoting the formation of conventional liquid emulsions.

Figure 4: Linseed oil nanoemulsions before and after long term stability experiments

Pseudo-ternary phase diagrams for the primary nanoemulsions were developed so that nanophasic regions could be identified for the optimization of formulations. From the phase diagram, it was clear that S_{mix} 1:2 was significantly reduced the interfacial tension on the w/o interface and a considerable self-emulsifying area appeared in the phase map. From the samples F1 to F7 we selected the optimum on the basis of average droplet size and drug entrapment. Among them, F5 contained the maximum drug entrapment in where the particle size is within range. The optimum concentration of oil phase and surfactant that was selected for the development of o/w nanoemulsion was 16.2 % and 21% respectively.

Nanoemulsions were characterized on the basis average droplet, size polydispersity index, pH, zeta potential, and percentage of drug entrapment.

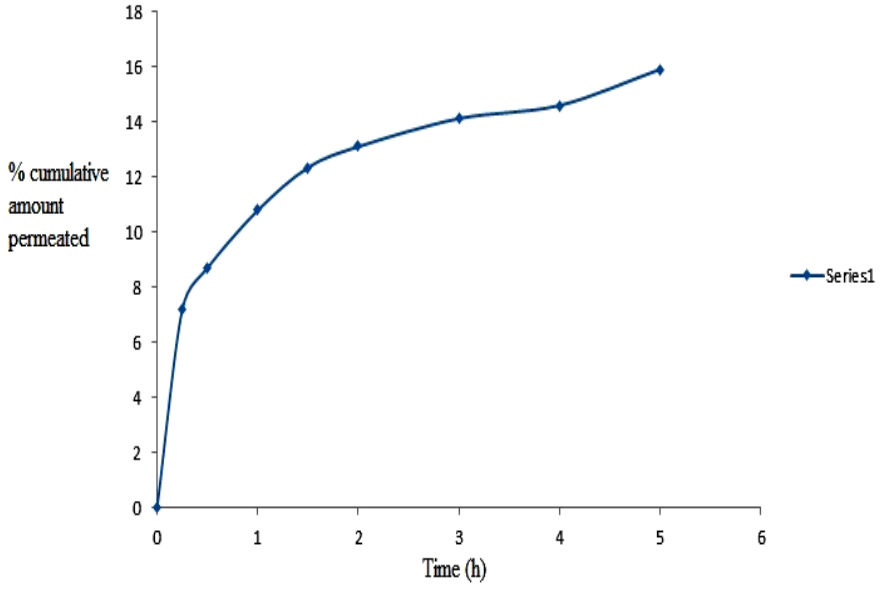

Figure 5: Ex vivo permeation of losartan from nanoemulsion at 37 °C.

And the value which was obtained was shown in Table 2. Texture and stability of the nanoemulsion depends on the distribution of droplet size is well established [14]. Dynamic light scattering was used to measurement the droplet size of the formulations, were shown in the table 2. The average droplet size of the nanoemulsion lied in a range of 287 to 350 nm. Polydispersity index is assessing of degree of homogeneity of particles which was measured here with the help of a zetasizer. Here we get a value for these formulations 0.35- 0.42 which was within a uniform range of pH was tested for all the formulation 2:8 to 8:2 containing losartan , and the result was obtained in between 4.60- 6.2. The pH of the optimum formulation (F5) 5.38±.01, which was present within the range (4.5- 5.5), which makes it promising for topical delivery [15].

Zeta potential can be measured by the ability of ionisation of the liquid surface and it is related with the particle electrokinetic properties [16]. The stability of these nanoemulsion formulations can be related with the droplet surface charge, which increase the stability by electrostatic repulsion, and these can be determine from the zeta potential value [17]. From the literature we know that the nanoemulsion is stable when the zeta potential value is greater the 25 mv [18]. In our study, we get the zeta potential value was -50 mv for the optimum formulation (F5), and the values for the other samples were shown in the table. With this characteristics, the permeation of drug improve across the skin occurs through electrostatic repulsion [19]. Drug entrapment percentage was observed to be highest in formulation F5, which was 78%, other value were also shown in the table 2.

Stability studies

In case of nanoemulsion development the major problem which demands the maximum attention is the stability problem [20]. It is intended that a product should be physically and chemically stable throughout its shelf life period. It has been observed in our study that polydispersity index droplet size, zeta potential of the samples did not change significantly through three freeze thaw cycles. When the nanoemulsion was exposed to centrifugal stress, no evidence of phase separation, creaming or cracking were observed. For long term stability studies, it was stored for 180 days in a well-sealed amber colored container in room temperature and no significant change was observed. It was found that the nanoemulsion formulation was stable in both stressed conditions and in a long time frame.

Ex-vivo skin permeation study

The transdermal permeation profile of losartan through goat ear skin from nanoemulsion was a typical steady state profile. After a lag time of 2 h for losartan nanonemulsion, a nearly linear relationship between cumulative amounts of drugs permeated versus time could be observed, indicating that the skin integrity was maintained throughout the experiment and the permeation rates were constants. A low permeation flux of 1.39 µg/cm2/h demonstrated that the system was ideal for slow release of losartan.

CONCLUSION

Conventional delivery of losartan by oral route possesses several drawbacks, such as it has low bioavailability due to high pass metabolism. Also it suffers from low half-life inside the biological system. In this present work, the transdermal delivery system of losartan through nanoemulsions was thoroughly investigated. Losartan-loaded nanoemulsions for transdermal delivery, containing linseed oil as the oil phase, Tween 80 as the surfactant, span 80 as the co-surfactant, were prepared. Stability studies performed under different stress and temperature conditions showed that the formulation could be stored for a long period

without exhibiting significant changes of its physicochemical properties. Ex vivo drug permeation studies showed that losartan permeation across the skin layers occurred at a slow rate while maintaining integrity of the goat ear skin. These results suggested that on linseed based nanoemulsion can used as promising carriers for transdermal delivery of losartan. However, future investigation on *in vivo* efficacy of delivered losartan must be performed before introducing the formulation for clinical applications.

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