Research & Reviews: Journal of Pharmaceutics and Nanotechnology e-ISSN: 2347-7857
p-ISSN: 2347-7849

Enhancement of Dissolution Rate and Bioavailability of Losartan by Self Micro Emulsifying Drug Delivery System: *In-Vitro* and *In-Vivo* Evaluation

Salunke Vaibhav*

Department of Pharmaceutics, Sharda College of Pharmacy, Ahmednagar, Maharashtra, India

Research Article

Received: 12-Sep-2022, Manuscript No. JPN-22-74357; **Editor assigned:** 14-Sep-2022, PreQC No. JPN-22-74357 (PQ); **Reviewed:** 28-Sep-2022, QC No. JPN-22-74357; **Revised:** 05-Jan-2023, Manuscript No. JPN-22-74357 (R); **Published:** 12-Jan-2023, DOI: 10.4172/2347-7857.11.1.001

***For Correspondence:** Salunke Vaibhav, Department of Pharmaceutics, Sharda College of Pharmacy, Ahmednagar, Maharashtra, India;
Email: salunkevaibhav92@gmail.com

Citation: Vaibhav S. Enhancement of Dissolution Rate and Bioavailability of Losartan by Self Micro Emulsifying Drug Delivery System: *In-Vitro* and *In-Vivo* Evaluation. RRJ Pharm Nanotechnol. 2023;11:001.

ABSTRACT

The objective of this study was to develop Self Micro Emulsifying Drug Delivery System (SMEDDS) to enhance the oral bio-availability of the poorly water soluble drug, Losartan. The influence of the oil, surfactant and co-surfactant types on the drug solubility and their ratios on forming efficient and stable SMEDDS were investigated in detail. The SMEDDS were characterized by morphological observation, droplet size and zeta potential determination, self-emulsification assessment, cloud point measurement, viscosity determination, refractive index, % transmittance, effect of pH of dilution media, drug content of SMEDDS of losartan and *in vitro* release study. The optimum formulation E1 consisted of Capmul MCM EP, Tween-80 and PEG-400 with S mix. Ratio of 1:1. *In vitro* release test showed a complete release of Losartan from SMEDDS in an approximately 1 hr. The absorption of Losartan from SMEDDS showed an increase in relative bioavailability compared with that of the marketed tablet formulation. Our studies demonstrated the promising use of SMEDDS for the delivery of Losartan by the oral route.

Keywords: Self-microemulsifying Drug Delivery System (SMEDDS); Losartan; Pseudoternary phase diagram; Nanoemulsion; Zeta potential

INTRODUCTION

Drugs are rarely administered in their pure forms and usually they have to be admixed with various kinds of adjuncts resulting into their transformation into 'dosage forms'. For the administration of the dosage forms, oral route is most preferred route but this route is frequently dependent upon the bioavailability of the active form of the drug.
Bioavailability is affected by the drug's physical-chemical properties, such as water solubility, oil solubility, its dissolution, pKa, stability, as well as its absorption, distribution, metabolism and excretion [1].
Together with the permeability, the solubility of a drug is a key determinant of its oral bioavailability. There have been certain drugs for which solubility has presented a challenge to the development of a suitable oral dosage form e.g. griseofulvin, digoxin, phenytoin, sulphathiazole and chloramphenicol [2].
Approximately 40% of new drug candidates have poor water solubility and the oral deliveries of such drugs are associated with implications of low bioavailability, high intra and inter subject variability and lack of dose proportionality. With the recent advent of high throughput screening of potential therapeutic agents, the numbers of poorly soluble drug candidates are increasing and the formulation of poorly soluble compounds for oral delivery now presents one of the most frequent and greatest challenges to formulation scientists in the pharmaceutical industry [3].

Enhanced dissolution/Solubilization

The presence of lipids in the GI tract stimulates gall bladder contractions, biliary and pancreatic secretions, including Bile Salts (BS), Phospholipids (PL) and Cholesterol (Ch). These products, along with the gastric shear movement form a crude emulsion and promote the solubilization of the co administered lipophilic drug. Surfactants present in the delivery system may also improve the solubilization of the lipophilic compound [4].

Self Microemulsifying Drug Delivery Systems (SMEDDS)

Self Emulsifying Drug Delivery Systems (SMEDDS) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants, or alternatively, one or more hydrophilic solvents and co-solvents/surfactants. Upon mild agitation followed by dilution in aqueous media, such as GI fluids, these systems can form fine oil in water (o/w) emulsions or microemulsions (SMEDDS). Self microemulsifying formulations spread readily in the gastro intestinal tract and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification. SEDDS typically produce emulsions with a droplet size between 100 and 300 nm while SMEDDS form transparent microemulsions with a droplet size of less than 50 nm. An additional advantage of SMEDDS over simple oily solutions is that they provide a large interfacial area for partitioning of the drug between oil and water. Thus, for lipophilic drug compounds that exhibit dissolution rate limited absorption, these systems may offer an improvement in the rate and extent of absorption and result in more reproducible blood time profiles and have been shown to enhance the oral bioavailability of lipophilic drugs such as cyclosporine, halofantrine, ontazolast and progesterone. The ease of dispersion and the very small particle size of the resultant colloidal microemulsion have been viewed as the principal reasons for their utility in the delivery of lipophilic drugs. Consequently, most of the commercially available lipid formulations are complex mixtures of lipids, surfactants, and cosolvents/cosurfactant constructed to improve drug solubility in the formulation (and therefore increase drug pay load) and also to maximize dispersion of the dose form on exposure of the capsule fill to the GI contents. When compared with emulsions, which are sensitive and metastable dispersed forms, SMEDDS are physically stable formulations that are easy to manufacture. Microemulsions are readily distinguished from normal emulsions by their transparency, their low viscosity, and more fundamentally their thermodynamic stability [5].

MATERIALS AND METHODS

As per the requirement selection of chemical non chemical material for preparation self-micro emulsifying drug delivery system from various sources such manufacturing company and chemical producer (Table 1).

Table 1. Name of material and its manufacturer.

Sr. No.	Material	Company/Manufacturer
1	Losartan	Zydus cadila Pvt Ltd Goa
2	Tween-80	Loba chemie Pvt Ltd Mumbai
3	Tween-20	Loba chemie Pvt Ltd Mumbai
4	Span 20	Loba chemie Pvt Ltd Mumbai
5	Span 80	Loba chemie Pvt Ltd Mumbai
6	PEG-600	Loba chemie Pvt Ltd Mumbai
7	PEG-400	Loba chemie Pvt Ltd Mumbai
8	PEG-200	Loba chemie Pvt Ltd Mumbai
9	Propylene glycol	Research lab, fine chem industries
10	Capmul MCM EP	Abitech corporation
11	Isopropyl myristate	Molychem Pvt Ltd
12	Soyabean oil	Adani Pvt Ltd
13	Oleic acid	Ozone international Mumbai
14	Methanol	Changshu Chemical China
15	Ethanol	Changshu Chemical China
16	Hydrochloric acid	Molychem Pvt Ltd
17	Distilled water	MESCOP, Sonai

All the above shown ingredients are used of analytical grade and free from any contamination [6].

Formulation and development of Self Microemulsifying Drug Delivery System (SMEDDS)

Many researchers in various literatures have reported the formulation techniques for microemulsion. These techniques include [7].

Selection of excipients for Self Micro Emulsifying Drug Delivery System (SMEDDS) formulation: Both long and medium chain triglyceride oils with different degrees of saturation have been tried for the design of SMEDDS formulations. Edible oils, which could represent the logical and preferred lipid excipients choice for the development of nanoemulsion, are not frequently screened due to their poor ability to dissolve large amounts of lipophilic drugs. Modified or hydrolyzed vegetable oils have been widely used since these excipients form good emulsification systems with a large number of surfactants approved for oral administration and exhibit better drug solubility properties. They offer formulative and physiological advantages and their degradation products resemble the natural end products of intestinal digestion. Novel semi synthetic medium chain derivatives, which can be defined as amphiphilic compounds with surfactant properties, are progressively and effectively replacing the regular medium chain triglyceride oils. The surfactant chosen must be able to lower interfacial tension to a very small value to aid dispersion process during the preparation of the nanoemulsion. Provide a flexible film that can readily deform around droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region for the desired nanoemulsion type. Safety is a major determining factor in choosing a surfactant as large amounts of surfactants may cause GI irritation. Non-ionic surfactants are less toxic than ionic surfactants. Non-ionic surfactants typically have lower CMCs than their ionic counterparts. O/W nanoemulsion dosage forms for oral or parenteral use based on nonionic surfactants are likely to offer *in-vivo* stability. An important criterion for selection of the surfactants is that the required HLB value to form o/w nanoemulsion is greater than. The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of single surfactant, usually necessitating the addition of a co-surfactant. The presence of co-surfactants decreases 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. Development of nanoemulsion systems for poorly water soluble drugs is critical. Components selected for the formulation should have the ability to solubilize the drug in high level to deliver the therapeutic dose of the drug in an encapsulated form. In general, excipients with higher solubilizing efficiency for drug are selected for formulation and development [8-14].

Drug solubility determination in the various oils, surfactants and co-surfactants: For formulating nanoemulsion drug delivery system the solubility of the drug in different oils is an essential step for the SMEDDS formulation. So before starting the phase diagram one must have to select the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is essential for the formulation of nanoemulsion drug delivery system.

Solubility study: 5 ml of selected vehicles (*i.e.* oil/surfactant/co-surfactant) were taken in a screw capped vials. Excess amount of Losartan was added to the mixture. The mixtures were shaken with magnetic stirrer at 25°C for 24 hrs. Once the equilibrium was reached each vial was centrifuged at 3000 rpm for 5 min, and the excess insoluble drug was discarded by filtration using membrane filter (0.45 µm, Whatman, Mumbai, India). The concentration of free drug was then quantified by the developed UV method (Table 2).

Table 2. List of vehicles (*i.e.* oil or surfactant or cosurfactant) used for solubility study.

Sr. no.	Vehicles
1	Soyabean oil
2	Olive oil
3	Oleic acid
4	Capmul MCM EP
5	Isopropyl myristate
6	Tween-80
7	Tween-20
8	PEG-400
9	PEG-200
10	PEG-600
11	Propylene glycol
12	Span-20, Span-80

Pseudoternary phase diagrams

Construction of phase diagram: Surfactant (Tween 80) and cosurfactant (PEG 400) were mixed (Smix) in different weight ratios (1:1, 1:2, 1:3, etc.). Capmul MCM EP oil was optimized as an oil phase based on the solubility study. For each phase diagram, oil (Capmul MCM EP) and specific Smix ratio were mixed thoroughly in different weight ratios *viz.*, 0.5:9.5, 1:9, 1.5:8.5, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Each ratio of oil and Smix was taken and titrated with water at 5% intervals and then mixed on a magnetic stirrer. The solutions were observed visually and were categorized into different phases:

- Transparent with good flow: oil/water Nanoemulsions (N)
- Transparent with slightly medium flow: Micro Emulsion (ME)
- Milky with good flow: Emulsion (E)
- Milky gel with good flow: Emulgel (M)

Based on the observation, phase diagram was constructed using CHEMIX school v3.51 Software with point A as oil, point B as Smix and point C as water. For each Smix ratio, a separate phase diagram was constructed and the area of nanoemulsion was shaded [15].

Preparation of SMEDDS formulations

The phase diagram construction Oleic acid, Tween-80, PEG 400 was used.

Based on the area of nanoemulsification from the phase diagrams, Smix ratio of 1:1, 2:1 and 3:1 were selected for the formulation development studies. In that first three formulations was selected from each ratio of Smix and having the ratio of oil and Smix was 0.5:9.5, 1:9, 1.5:8.5 respectively. SMEDDS formulations were prepared using Tween 80 and PEG 400 as surfactant and co-surfactant with Smix ratio of 1:1, 2:1 and 3:1 (Table 3). The weight of the formulation was kept approx 10 ml. Level of losartan in all the formulation was kept constant (12.5 mg/ml). Losartan was accurately weighed and placed in a glass vial with the respective required quantity of oleic acid (oil). The components were mixed by gentle stirring and vortex mixing. Respective quantity of surfactant and cosurfactant were added to the vial and mixed by magnetic stirrer. The mixture was stored at room temperature [16].

Table 3. Developed F1-F9 formulation with their composition.

Sr. no.	Ratio	Oil	Smix	Batch
1	0.5:9.5	0.15	2.85	F1
2	01:09	0.3	2.7	F2
3	1.5:8.5	0.45	2.55	F3
4	0.5:9.5	0.15	2.85	F4
5	01:09	0.3	2.7	F5
6	1.5:8.5	0.45	2.55	F6
7	0.5:9.5	0.15	2.85	F7
8	01:09	0.3	2.7	F8
9	1.5:8.5	0.45	2.55	F9

Preparation of SMEDDS formulations

The phase diagram construction Capmul MCM EP oil, Tween-80, PEG 400 was used. Based on the area of nanoemulsification from the phase diagrams, Smix ratio of 1:1, 2:1 and 3:1 were selected for the formulation development studies. In that first three formulations was selected from each ratio of Smix and having the ratio of oil and Smix was 0.5:9.5, 1:9, 1.5:8.5 respectively. SMEDDS formulations were prepared using Tween 80 and PEG 400 as surfactant and co-surfactant with Smix ratio of 1:1, 2:1 and 3:1 (Table 4). The weight of the formulation was kept approx 10 ml. Level of losartan in all the formulation was kept constant (12.5 mg/ml). Losartan was accurately weighed and placed in a glass vial with the respective required quantity of Capmul MCM EP oil. The components were mixed by gentle stirring and vortex mixing. Respective quantity of surfactant and cosurfactant were added to the vial and mixed by magnetic stirrer. The mixture was stored at room temperature [17].

Table 4. Developed E1-E9 formulation with their composition.

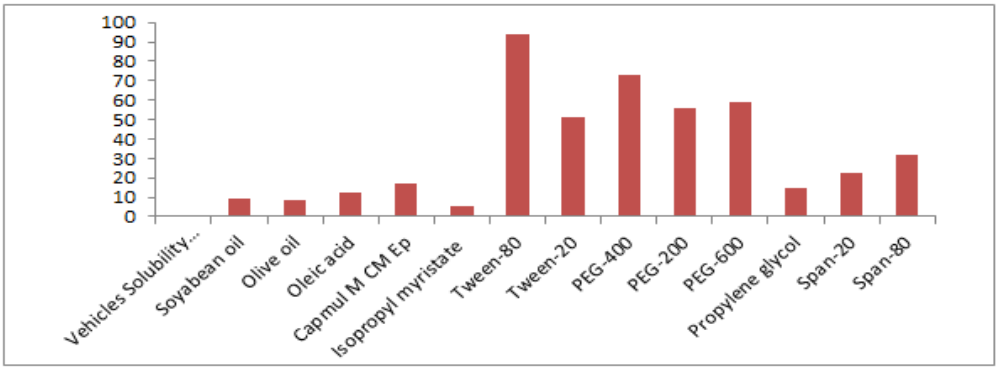
Sr.no.	Ratio	Oil	Smix	Batch
1	0.5:9.5	0.15	2.85	E1
2	01:09	0.3	2.7	E2
3	1.5:8.5	0.45	2.55	E3
4	0.5:9.5	0.15	2.85	E4
5	01:09	0.3	2.7	E5
6	1.5:8.5	0.45	2.55	E6
7	0.5:9.5	0.15	2.85	E7
8	01:09	0.3	2.7	E8
9	1.5:8.5	0.45	2.55	E9

RESULTS AND DISCUSSION

Solubility study

The results of solubility studies of Losartan in various oils, cosurfactant, and surfactants. In order to achieve optimum drug loading, solubility study was aimed to identify suitable SMEDDS components that possess good solubilizing capacity for Losartan. Among the various oils tested Capmul MCM EP (17.35 ± 0.3060 mg/ml) showed higher solubility for Losartan and Tween 80 (93.6 ± 0.4414 mg/ml) and PEG 400 (72.9 ± 0.24333 mg/ml) exhibited the higher solubility for Losartan among the various surfactant and cosurfactant tested. Based on the solubility data, Capmul MCM EP was selected as oil phase, Tween 80 as surfactant, PEG 400 as co-surfactant for formulating SMEDDS of Losartan as these solvents showed higher solubility. In addition, synthetic oils have been reported to form good emulsification [18] (Figures 1-7).

Figure 1. Graph of vehicles solubility.



Construction of phase diagram

The phase diagram construction Oleic acid, Tween-80, PEG 400 was used.

Figure 2. Phase diagrams of Oleic acid, Tween 80, PEG 400 systems indicating nanoemulsion existence region with Tween 80 and PEG 400 ratio of 1:1.

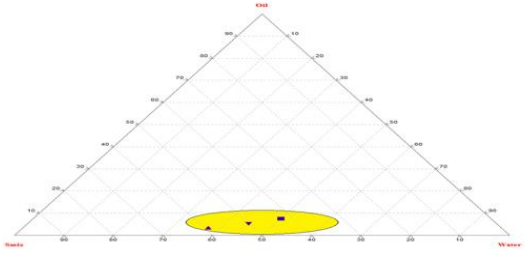


Figure 3. Phase diagrams of Oleic acid, Tween 80, PEG 400 systems indicating nanoemulsion existence region with Tween 80 and PEG 400 ratio of 2:1.

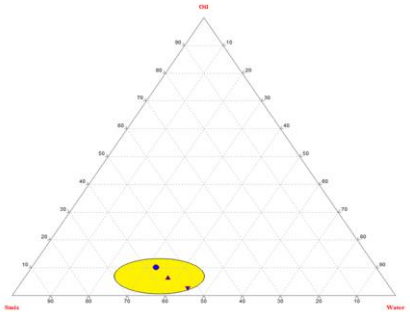
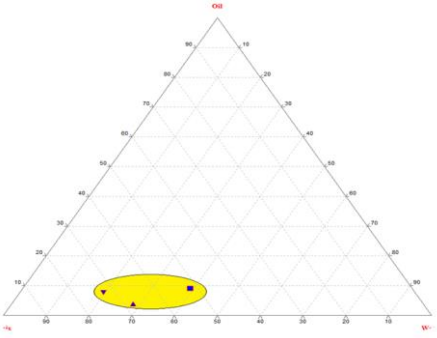


Figure 4. Phase diagrams of Oleic acid, Tween 80, PEG 400 systems indicating nanoemulsion existence region with Tween 80 and PEG 400 ratio of 3:1



Construction of phase diagram

The phase diagram construction Capmul MCM EP oil, Tween-80, PEG 400 was used.

Figure 5. Phase diagrams of Capmul MCM EP, Tween 80, PEG 400 systems indicating nanoemulsion existence region with Tween 80 and PEG 400 ratio of 1:1.

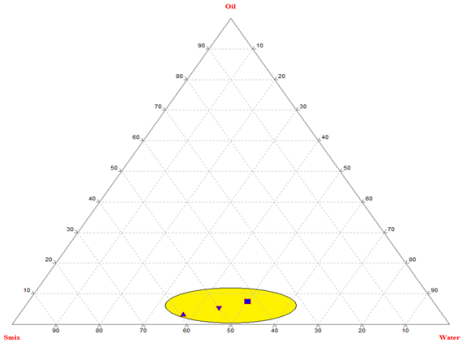


Figure 6. Phase diagrams of Capmul MCM EP, Tween 80, PEG 400 systems indicating nanoemulsion existence region with Tween 80 and PEG 400 ratio of 2:1.

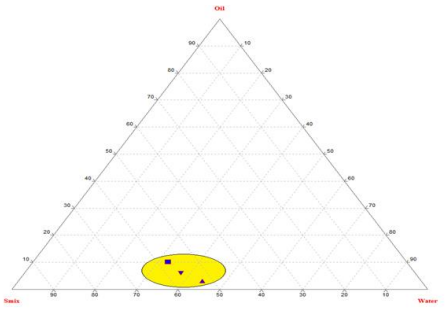
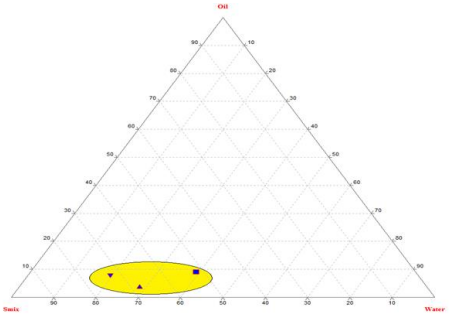


Figure 7. Phase diagrams of Capmul MCM EP, Tween 80, PEG 400 systems indicating nanoemulsion existence region with Tween 80 and PEG 400 ratio of 3:1.



Drug content of SMEDDS of losartan

The drug was shown in Tables 5 and 6 all the F1-F9 and E1-E9 formulation shows the drug content above 90%. As compare to F1 formulation (96.48 ± 0.2800) the E1 formulation which shows the greater % drug release (98.48 ± 0.2946).

Table 5. Drug content of F1-F9 formulation.

Sr.no.	Formulation code	% drug content
1	F1	96.48 ± 0.2800
2	F2	93.54 ± 0.9539
3	F3	95.23 ± 0.3907
4	F4	90.77 ± 0.1692
5	F5	94.98 ± 1.149
6	F6	89.30 ± 0.8544

7	F7	95.65 ± 1.015
8	F8	92.58 ± 0.5033
9	F9	96.06 ± 0.5000
Mean ± SD; n=3		

Table 6. Drug content of E1-E9 formulation.

Sr.no.	Formulation code	% drug content
1	E1	98.48 ± 0.2946
2	E2	96.55 ± 0.7422
3	E3	94.86 ± 0.1709
4	E4	94.61 ± 0.1206
5	E5	91.62 ± 0.2610
6	E6	91.48 ± 0.2623
7	E7	95.54 ± 0.2335
8	E8	95.39 ± 0.8361
9	E9	95.81 ± 0.6393
Mean ± SD; n=3		

Microemulsion droplet size

There is a relationship between the droplet size and the concentration of the surfactant being used. In some cases, increasing the surfactant concentration could lead to droplets with smaller mean droplet size. This could be explained by the stabilization of the oil droplets as a result of the localization of the surfactant molecules at the oil–water interface. On the other hand, in some cases, the mean droplet size may increase with increasing surfactant concentrations. This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase [19].

The mean droplet size and PDI for all the SMEDDS have been summarized in Table 7 and Figure 8. Polydispersity is the ratio of standard deviation to the mean droplet size. This signifies the uniformity of droplet size within the formulation. The higher the value of Polydispersity, the lower is the uniformity of the droplet size in the formulation. The formulation E1 and E2 which have showed the less particle size as compare to the F1 and F2 formulation. This was because the polydispersive index of the E1 and E2 formulation was less as compared to the F1 and F2 formulation.

Figure 8. Microemulsion droplet size of E1 formulation.

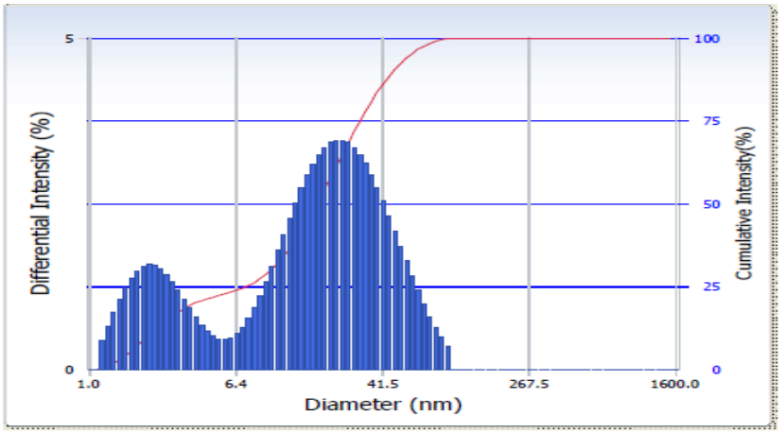


Table 7. Microemulsion E1 Particle size cumulants results.

Sr.no.	Cumulants results	Observation
1	Diameter (d)	69.0 nm
2	Polydispersive index (P.I.)	0.377

3	Diffusion const. (D)	7.143 e-008 (cm^2/sec)
Measurement condition		
4	Temperature	25.1 (^{0}c)
5	Diluent name	Water
6	Refractive index	1.3328
7	Viscosity	0.8858 (cP)
8	Scattering intensity	8554 (cps)

Transmission Electron Microscopy (TEM)

The microemulsion formulation samples were examined by electron microscopy to study the particle shape and verify the droplet size determined by light scattering analysis. The shape of particles may have a significant impact on the performance of the formulation. Moreover, the study of droplet shape provides a check on the validity of the size measurement and data analysis which assume spherical droplets.

The TEM and cryo FESEM micrographs of the nanoemulsion formulations were shown in Figures 9 and 10 to illustrate their microstructure. These structures were considered to be o/w nanoemulsion because they have been formed at high water content (87 wt. % water). This supported by the results from electrical conductivity measurement. From the Figure 9 displayed, the microstructures observed are discrete spherical droplets. All of the different compositions of microemulsion formulation show similar structures (Tables 8-10). In the TEM micrographs, the droplets were highly uniform with an average droplet diameter smaller than 50 nm (Figures 11-13). These diameters of droplet observed in TEM are in good accordance with the hydrodynamic diameter values measured by DLS. TEM image exhibited that the particle were discrete, non-aggregated, homogenously dispersed and nearly spherical in shape [20].

Figure 9. Microemulsion droplet size of E2 formulation.

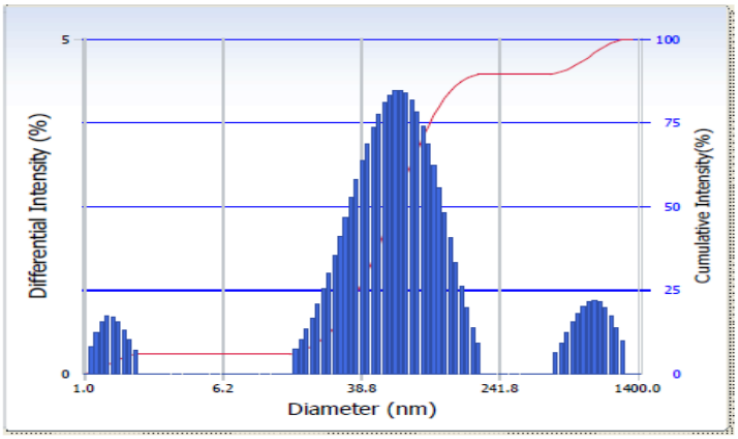


Table 8. Microemulsion E2 particle size cumulants results.

Sr. no.	Cumulants results	Observation
1	Diameter (d)	103.9 nm
2	Polydispersive index (P.I.)	0.128
3	Diffusion const. (D)	4.732 e-008 (cm^2/sec)
Measurement condition		
4	Temperature	25.1 (^{0}c)
5	Diluent name	Water
6	Refractive index	1.3328
7	Viscosity	0.8878 (cP)
8	Scattering intensity	9473 (cps)

Figure 10. Microemulsion droplet size of F1 formulation.

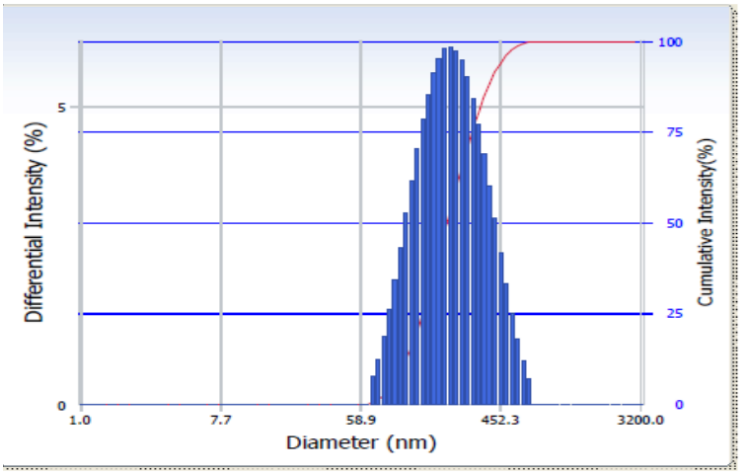


Table 9. Microemulsion F1 Particle size cumulants results.

Sr.no.	Cumulants results	Observation
1	Diameter (d)	185.2 nm
2	Polydispersive index (P.I.)	0.218
3	Diffusion const. (D)	2.657 e-008 (cm^2/sec)
Measurement condition		
4	Temperature	25 (^{0}c)
5	Diluent name	Water
6	Refractive index	1.3328
7	Viscosity	0.8878 (cP)
8	Scattering intensity	8104 (cps)

Figure 11. Microemulsion droplet size of F2 formulation.

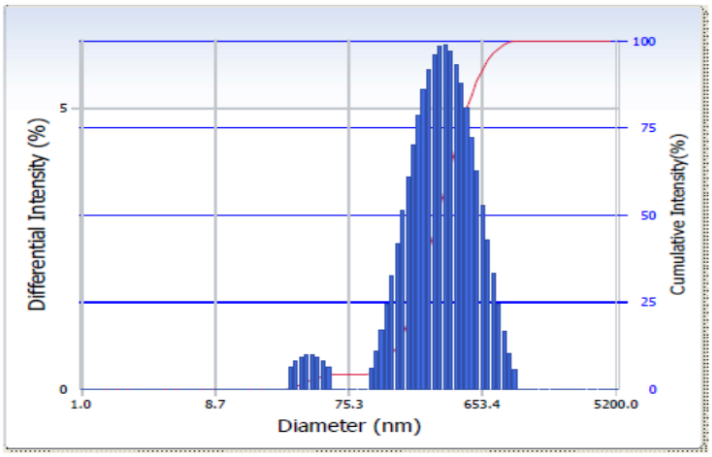


Table 10. Microemulsion F2 Particle size cumulants results.

Sr. no.	Cumulants results	Observation
1	Diameter (d)	267.1 nm
2	Polydispersive index (P.I.)	0.284
3	Diffusion const. (D)	1.84 e-008 (cm^2/sec)
Measurement condition		
4	Temperature	25 (^{0}c)

5	Diluent name	Water
6	Refractive index	1.3328
7	Viscosity	0.8858 (cP)
8	Scattering intensity	10209 (cps)

Figure 12. TEM of E1 formulation.

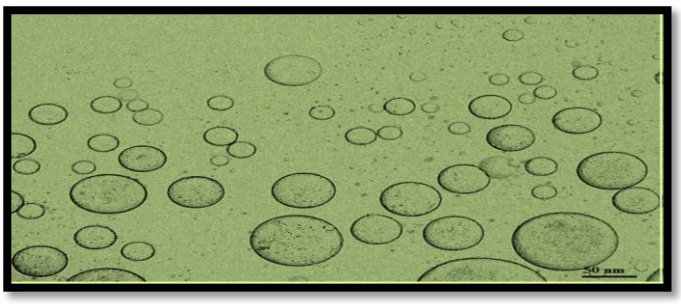
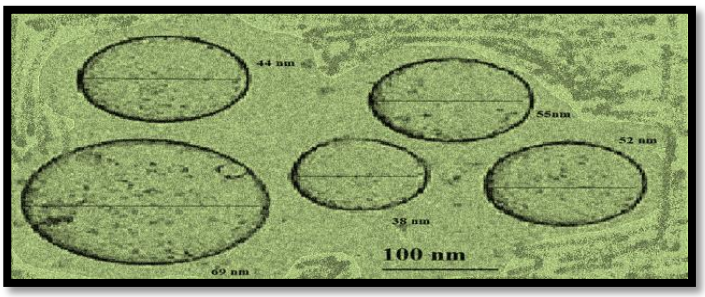


Figure 13. TEM of E1 formulation size measurement at 100 nm.



The *in-vitro* dissolution study

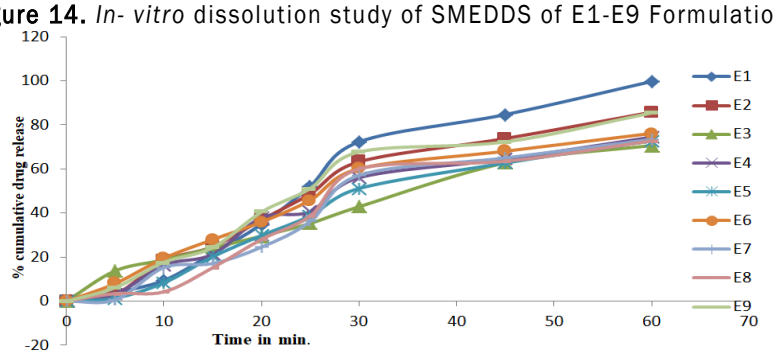
The SMEDDS and plain losartan were carried out using USP type-II dissolution test apparatus in 0.1 N HCL solutions at 37 ± 2^{0}C with 50 rpm rotating speed. Samples of 1 mL were withdrawn at regular time interval of 5, 10, 15, 20, 25, 30, 45, and 60 min. and filtered using 0.45 µm filter. An equal volume of respective dissolution medium was added to maintain the volume constant. Drug content from sample was analyzed using UV-spectrophotometer at 221 nm. All measurements were done in triplicate from three independent samples (Table 11 and Figure 14).

Table 11. *In-vitro* dissolution study of SMEDDS of E1-E9 and SMEDDS formulation.

Sr. no.	% Cumulative drug release									
	Time in min.	E1	E2	E3	E4	E5	E6	E7	E8	E9
1	0	0	0	0	0	0	0	0	0	0
2	5	3.91 ± 0.0152	3.14 ± 0.0152	13.76 ± 0.0208	3.25 ± 0.0100	1.36 ± 0.0100	8.007 ± 0.0281	0.591 ± 0.0010	3.46 ± 0.0305	6.12 ± 0.4996
3	10	9.45 ± 0.0115	18.3 ± 0.1528	18.75 ± 0.0208	16.42 ± 0.0200	8.34 ± 0.0115	19.52 ± 0.0300	15.31 ± 0.0152	4.24 ± 0.0321	17.64 ± 0.1929
4	15	21.64 ± 0.0208	24.75 ± 0.0115	24.2 ± 0.0200	21.31 ± 0.0264	20.19 ± 0.0100	27.96 ± 0.0100	17.1 ± 0.2082	15.21 ± 0.0450	24.3 ± 0.3606
5	20	34.94 ± 0.0152	36.95 ± 0.0152	29.54 ± 0.0100	37.94 ± 0.0200	29.84 ± 0.0100	35.96 ± 0.4239	24.53 ± 0.2631	28.06 ± 0.4500	40.49 ± 0.2916
6	25	52.25 ± 0.0264	48.72 ± 0.0208	35.33 ± 0.0100	40.64 ± 0.0208	38.73 ± 0.0152	45.85 ± 0.4157	36.18 ± 0.0907	38.72 ± 0.0964	51.16 ± 0.4202
7	30	72.34 ± 0.0251	63.28 ± 0.0100	42.89 ± 0.0208	56.07 ± 0.0100	51.17 ± 0.0152	60.18 ± 0.4734	57.14 ± 0.0808	60.02 ± 0.5658	67.73 ± 0.2219
8	45	84.71 ± 0.0230	73.86 ± 0.0208	62.76 ± 0.0100	64.54 ± 0.0152	62.74 ± 0.0100	68.1 ± 0.4509	65.06 ± 0.0360	63.51 ±	72.32 ±

									0.3017	0.3765
9	60	99.75 ± 0.0288	85.79 ± 0.0100	70.698 ± 0.0264	74.47 ± 0.0152	72.77 ± 0.0152	76.15 ± 0.4744	73.44 ± 0.0808	72.77 ± 0.1735	85.46 ± 0.2786
Mean ± SD; n=3										

Figure 14. *In- vitro* dissolution study of SMEDDS of E1-E9 Formulation.



In-vivo study

Pharmacokinetic analysis: Plasma samples collected from the rabbits were analyzed using developed reverse phase HPLC method the drug plasma concentration values were determined from the calibration curve (Tables 12-17 and Figures 15-19).

Table 12. Peak area of calibration curve.

Sr. No	Retention time	Peak Area (µV/sec)	% area	Symmetric factor	NTP
1	2.733	1212647	100	1.327	606

Figure 15. Chromatogram of Losartan 25 µg/ml.

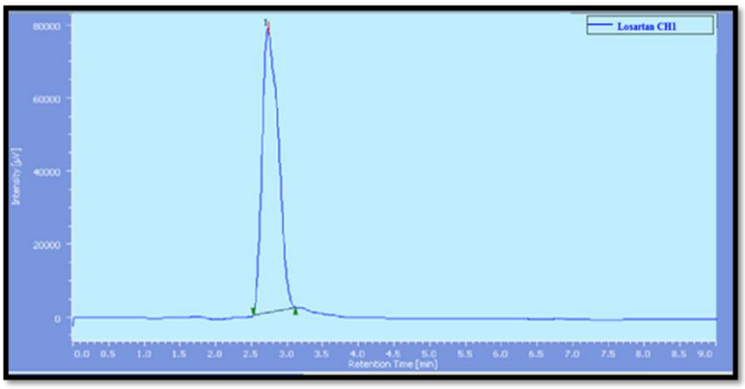


Table 13. HPLC data for the calibration curve of Losartan.

Sr.no.	Concentration (µg/ml)	Area
1	5	240123
2	10	489561
3	15	721365
4	20	1084561
5	25	1212647

Figure 16. Calibration curve for Losartan.

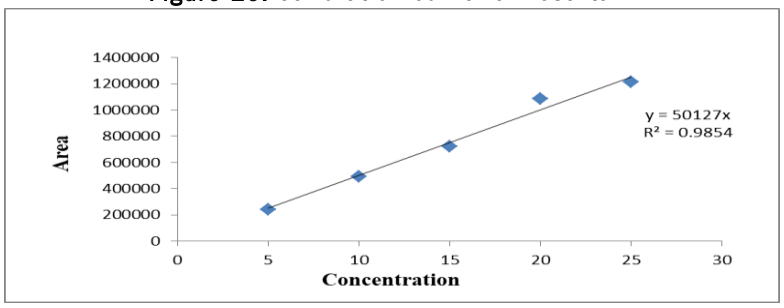


Table 14. The drug plasma concentration values of the SMEDDS.

Sr. No	Retention time	Peak Area (µV/sec)	% area	Symmetric factor	NTP
1	2.673	150256	100	1.803	133

Figure 17. Chromatogram SMEDDS E1 formulation.

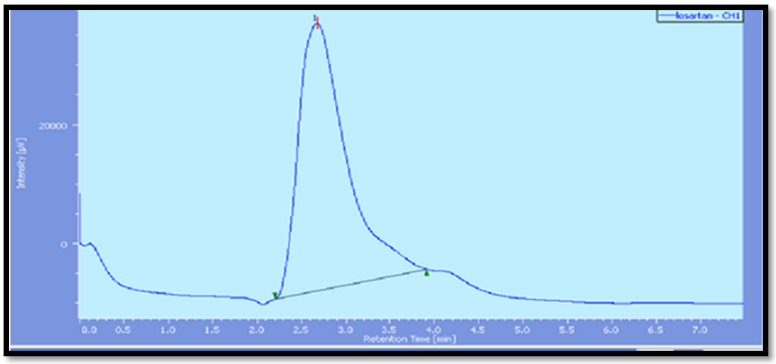


Table 15. Chromatogram pure drug values.

Sr. No	Retention time	Peak Area (µV/sec)	% area	Symmetric factor	NTP
1	2.2	112396	100	1.236	127

Figure 18. Chromatogram Pure drug formulation.

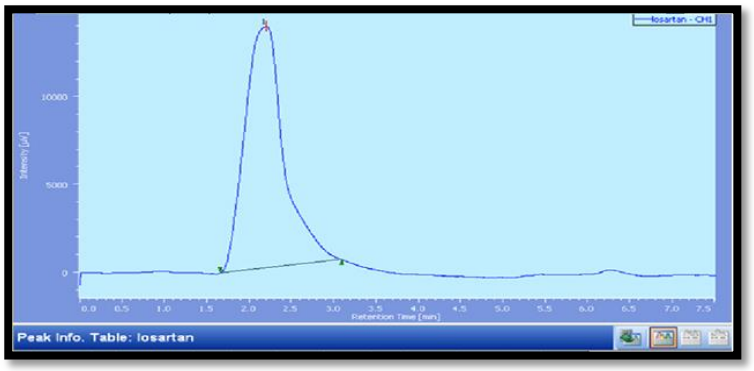


Table 16. Chromatogram marketed drug values.

Sr. No	Retention time	Peak Area (µV/sec)	% area	Symmetric factor	NTP
1	2.253	100231	100	1.136	130

Figure 19. Chromatogram marketed drug formulation.

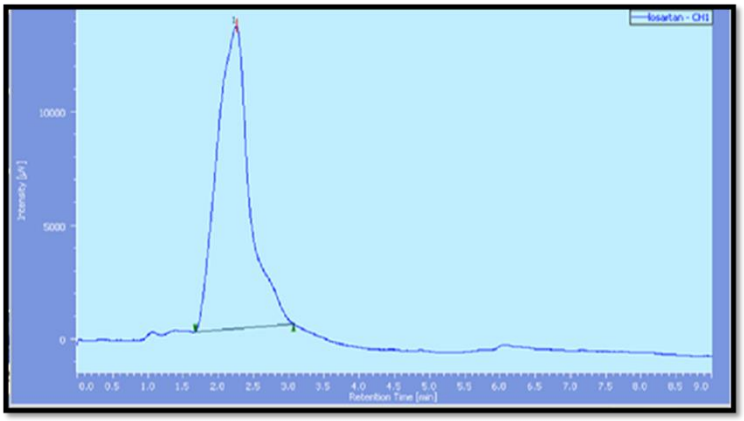


Table 17. *In-Vivo* bioavailability study data.

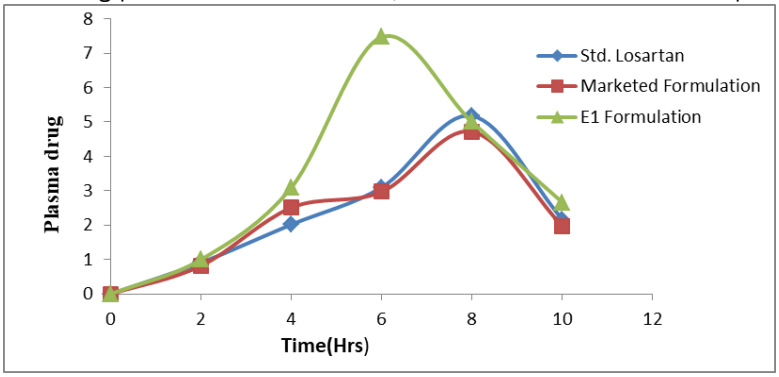
Sr. No.	Time interval (Hrs.)	Standard Losartan		Marketed formulation		Optimized batch E1	
		Peak area	Conc. µg/ml	Peak area	Conc. µg/ml	Peak area	Conc. µg/ml
1	2	45687	0.91 ± 0.02646	41246	0.82 ± 0.02517	50269	1.002 ± 0.1517
2	4	101467	2.02 ± 0.2411	125964	2.51 ± 0.04509	155769	3.10 ± 0.2082
3	6	155769	3.10 ± 0.1528	148920	2.97 ± 0.3958	374586	7.47 ± 0.04041
4	8	259870	5.18 ± 0.07937	237021	4.72 ± 0.06557	250732	5.00 ± 0.1528
5	10	109240	2.17 ± 0.06807	99284	1.97 ± 0.6807	133680	2.66 ± 0.07211
Mean ± SD; n=3							

In-vivo bioavailability data shows plasma drug conc. at time interval in that the standard Losartan shows the C_{max} at 5.18 µg/ml at 8 hrs. and marketed formulation shows the C_{max} at 4.72 µg/ml. as compare to std. Losartan and marketed formulation, optimized batch E1 shows the increase C_{max} 7.47 µg/ml. at 6 hrs (Table 18 and Figure 20).

Table 18. Comparative study of the pharmacokinetic parameters of optimized batch, Std. Losartan, and marketed formulation.

Sr.No.	Pharmacokinetic parameter	Std. Losartan	Marketed formulation	Optimized batch E1
1	T_{max}	10.73	8.76	7.14
2	C_{max}	5.18 µg/ml	4.72 µg/ml	7.47 µg/ml
3	AUC	24.31 µg.hr./ml	23.89 µg.hr./ml	36.52 µg.hr./ml
4	MRT	22.73 hr.	17.92 hr.	14.38 hr.

Figure 20. Plasma drug profile of Std. Losartan, marketed formulation and optimized batch E.



CONCLUSION

Our studies demonstrated the promising use of SMEDDS for the delivery of Losartan by the oral route. The results of drug release and oral bioavailability of losartan SMEDDS were compared with marketed formulation. The selected formulation enhanced the oral bioavailability of losartan by 1.49 folds than the marketed formulation. The results of solubility studies of Losartan in various oils, cosurfactant, and surfactants. In order to achieve optimum drug loading, solubility study was aimed to identify suitable SMEDDS components that possess good solubilizing capacity for Losartan.

REFERENCES

1. Aungst BJ, et al. Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism. J Pharm Sci. 1993;82:979–986.
2. Burcham DL, et al. Improved oral bioavailability of the hypocholesterolemic in dogs following oral dosing in oil and glycol solutions. Biopharm Drug Dispos. 1997;18:737–742.
3. Chris de Smidt P, et al. Intestinal absorption of penclomedine from lipid vehicles in the conscious rat: contribution of emulsification *vs*. digestibility, Int J Pharm. 2004;270:1-2.
4. Constantinides PP, et al. Lipid microemulsions for improving drug dissolution and oral absorption: Physical and biopharmaceutical aspects. Pharm Res. 1995;12:1561–1572.
5. Charman WN, et al. Lipid vehicle and formulation effects on intestinal lymphatic drug transport. 1st Edition. Routledge. United States. 2019:113-179.
6. Craig DQM, et al. An investigation into the physicochemical properties of self-emulsifying systems using low frequency dielectric spectroscopy, surface tension measurements and particle size analysis. Int J Pharm. 1993;96:147–155.
7. Dabros T, et al. Emulsification through area contraction. J Colloid Interface Sci. 1999;210:222–224.
8. Dahan A, et al. Rationalizing the selection of oral lipid based drug delivery systems by an *in vitro* dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. J Control Release. 2008;129:1-10.
9. Embleton JK, et al. Structure and activity of gastrointestinal lipases. Adv Drug Deliv Rev. 1997;25:15-32.
10. Groves MJ, et al. Phase studies of mixed phosphate surfactants, n-hexane and water. J Pharm Pharmacol. 2004;26:616–623.
11. Gursoy NR, et al. Self-Emulsifying Drug Delivery Systems (SEDDS) for improved oral delivery of lipophilic drugs, Biomed Pharmacother. 2004;58:173-182.
12. Hauss DJ, et al. Lipid based delivery systems for improving the bioavailability and lymphatic transport of a poorly water-soluble LTB4 inhibitor. J Pharm Sci. 1998;87:164-169.
13. Humberstone AJ, et al. Lipid based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev. 1997;25:103-128.
14. Khoo SM, et al. Intestinal lymphatic transport is the primary route for halofantrine after oral postprandial administration. Pharm Res. 1999;1.
15. Kommuru TR, et al. Self-Emulsifying Drug Delivery Systems (SEDDS) of coenzyme Q10: Formulation development and bioavailability assessment. Int J Pharm. 2001;212:233–246.
16. Nikkila T, et al. Mechanisms of absorption enhancement by medium chain fatty acids in intestinal epithelial $Caco_2$ monolayers. J Pharmacol Exp Ther. 1995;275:958–964.
17. Leuner C, et al. Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm. 2000;50:47-60.
18. Loebenberg R, et al. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur J Pharm Biopharm. 2000;50:3-12.
19. Loftsson T, et al. Role of cyclodextrin in improving oral drug delivery. Am J Drug Deliv. 1999;2:1-10.
20. Makhey VD, et al. Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells. Pharm Res. 1998:1160-1167.