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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and Evaluation of Self-Microemulsifying Drug Delivery Systems (SMEDDS) Of Irbesartan and Their Pharmacokinetic Studies

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ABSTRACT

The main purpose of this investigation was to prepare self-microemulsifying drug delivery system (SMEDDS) for enhancement of oral bioavailability of a poorly water soluble drug Irbesartan (IRB), a BCS class II drug by improving its dissolution rate. Self-Emulsifying Drug Delivery Systems (SEDDS) of IRB were formulated using cinnamon essential oil as the oil phase, Gelucire 44/14 as the surfactant and Transcutol HP as co-surfactant. Drug-excipient interactions were studied by FTIR spectroscopy. The formulations were evaluated for its self-emulsifying ability, clarity, and stability of the aqueous dispersion after 48 h and the phase diagram was constructed to optimize the system. Selected formulations were characterized in terms of droplet size distribution, zeta potential, cloud point and were subjected to in vitro drug release studies. The bioavailability of optimized formulation was assessed in New Zealand white rabbits. By considering smaller droplet size, higher zeta potential and faster rate of drug release the formulation TF9 was chosen as optimized SMEDDS formulations. TF9 was robust to different pH media and dilution volumes, remained stable after three cooling-heating cycles and after stored at 4 °C and 25 °C for 3 mo without showing a significant change in droplet size. The pharmacokinetic study in rabbits showed SMEDDS have significantly increased the C_{max} and area under the curve (AUC) of IRB compared to suspension ($P<0.05$). SMEDDS can be an effective oral dosage form for enhancing aqueous solubility and improving oral bioavailability of poorly water soluble drugs.

Keywords: *Irbesartan, SMEDDS, Cinnamon essential oil, Gelucire 44/14, Transcutol HP, aqueous solubility, dissolution rate, bioavailability*

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INTRODUCTION

The oral route of drug administration has always been preferred due to its simplicity, patient convenience, compliance, accurate dosage and low cost of production [1]. A most essential requirement for oral absorption is that the drug must present in a solubilized state prior to passage across the gastrointestinal (GI) membrane. There are various pharmaceutical and physiological factors which affect the gastrointestinal absorption as well as the bioavailability of the drugs [2]. The reasons which contributed to poor oral bioavailability include less aqueous solubility, inadequate lipophilicity, and gastrointestinal degradation of the drug, presystemic metabolism and P-glycoprotein (Pgp) efflux of some drugs [3, 4]. Though continuous attempts are undertaken to minimize the solubility problems, approximately 40% of the currently marketed immediate-release (IR) oral drugs [5] and up to 75% of compounds currently under development have been categorized to be poorly water soluble (<100 µg/ml) [6, 7]. These low soluble drugs exhibit poor bioavailability where dissolution is the rate-limiting step [8]. The various strategies such as solid dispersions [9], complexation with cyclodextrin [10], micronization, nanoparticles, permeation enhancers [11], cocrystal formation [12] and lipid-based formulations [13] have been reported in the literature. In recent years much attention has been focused on lipid-based formulations [14] with emphasis on self-emulsifying drug delivery systems (SEDDS) to improve oral bioavailability of lipophilic drugs [15]. Self-micro emulsifying drug delivery systems (SMEDDS) are defined as the isotropic mixtures of oil, surface active agents and co-surfactant (CoS) in which a particular drug is present in the dissolved state and such system rapidly form fine oil-in-water (o/w) microemulsions when introduced into the aqueous medium under mild agitation [16].

Irbesartan, a BCS class II drug [17] is widely used in the treatment of hypertension. The drug is practically insoluble in water and shows dissolution rate limited bioavailability. Therefore the aim of present study was to improve the oral bioavailability of IRB via SMEDDS approach.

The conventional SMEDDS include a relatively large amount of surfactants (>70%), which may induce GI irritation and side-effects [18]. In order to achieve a safe and efficient delivery system for the poor oral bioavailability drugs, the investigation was aimed to design a novel SMEDDS with a high proportion of cinnamon oil (an essential oil as the carrier for lipophilic drugs).

The aim of the present investigation was to develop and characterize the optimized formulation of SMEDDS containing Irbesartan and to assess its bioavailability in the rabbits.

In the present investigation, essential oil was used to replace part of the surfactant for reducing the potential toxicity of the formulation. It was observed that high essential oil containing SMEDDS formulations possess excellent self-emulsifying property, stability and suitable *in vitro* drug release profile, without affecting the drug loading capacity.

Studies on mean particle sizes of microemulsions were conducted by dynamic light scattering (DLS) technique. The *in vitro* release profiles of Irbesartan from SMEDDS and the prepared IRB suspension were compared. Various pharmacokinetics parameters were investigated for optimized SMEDDS and prepared IRB suspension and relative oral bioavailability of IRB was assessed.

MATERIAL AND METHODS

Materials

Irbesartan was received as a gift sample from Dr. Reddy's Laboratory (Hyderabad, India). Gelucire 44/14 (Lauroyl Polyoxyl-32 glycerides) and Transcutol HP (Diethylene glycol monoethyl ether) were kindly provided by Gattefosse India Pvt. Ltd. (Mumbai). Anise oil, cinnamon oil, and lemon essential oil were purchased from Genuine Chemicals Co. (Mumbai, India). Oleic acid ((9Z)-Octadec-9-enoic acid), polyethylene glycol 400 and propylene glycol were purchased from Molychem (Mumbai, India). Tween (R) 80 (Polyoxyethylene (20) sorbitan monooleate) and olive oil were purchased from Loba Chemie Pvt. Ltd. (Mumbai, India). Glycerol was purchased from Sisco Research Laboratory (Mumbai, India), Cremophore RH40 and dialysis membrane (DM-50) were purchased from Himedia (Mumbai, India). All the excipients and reagents were of analytical grade and double distilled water was freshly prepared whenever required throughout the study.

Methods

Solubility study

Solubility studies for the drug in different vehicles were carried by placing an excess amount of Irbesartan in screw-capped vials containing 2 ml of vehicle. To facilitate the solubilization, the suspensions were heated on a water bath at 40 °C and then stirred using vortex mixer. The suspensions were continuously agitated on a water bath shaker for 48 h at ambient temperature until equilibrium was reached. Then the samples were centrifuged at 3000 rpm for 15 min and the supernatant was taken, filtered through the membrane filter (0.45 µm, 13 mm, Whatman, USA). The filtrates were suitably diluted with methanol and analyzed by UV-Visible spectrophotometer (Shimadzu, Japan) for the dissolved drug at 294 nm [19].

Surfactant and oil miscibility

The oil and surfactant in the ratio of 1:1 were shaken at 40 °C in 5 ml transparent glass vials. The miscibility was monitored optically and considered to be good when the mixture was transparent.

Fourier transform infrared spectroscopy

To investigate any possible interaction between the drug and utilized excipients, FTIR spectroscopy was used [20]. The IR spectra of pure drug and that of SEDDS were recorded by using FTIR Spectrometer (FTIR-8400S, Shimadzu, Japan). Sample preparation includes mixing a small quantity of the sample with Nujol and was placed in the FTIR sample holder. The IR spectrum was recorded from regions of 4000 cm^{-1} to 400 cm^{-1}.

Preparation of SEDDS

A series of SEDDS formulations were prepared with varying ratios of oil (30–70%), surfactant (20–69%) and co-surfactant (4–27%) as shown in table 1. The surfactant and co-surfactant (S/CoS) were used at the ratio of 2:1, 4:1 and 6:1. A single dose of IRB (20 mg/ml) was incorporated in all formulations. The formulations were developed by dissolving the drug in oil followed by addition of surfactant previously heated to 50 °C and co-surfactant in glass vials. The resultant mixtures were stirred continuously by vortex mixing and heated at 50 °C to obtain a homogeneous isotropic mixture. The SEDDS formulations were stored at ambient temperature until further use.

Table 1: Composition of SEDDS formulations of Irbesartan (% v/v)

Formulation	Ingredients (% v/v)		
	Cinnamon oil	Gelucire 44/14	Transcutol HP
TF1	20	53.3	26.6
TF2	20	64	16
TF3	20	68.5	11.4
TF4	30	46.6	23.3
TF5	30	56	14
TF6	30	60	10
TF7	40	40	20
TF8	40	48	12
TF9	40	51.4	8.5
TF10	50	33.3	16.6
TF11	50	40	10
TF12	50	42.6	7.4
TF13	60	26.6	13.4
TF14	60	32	8
TF15	60	34.3	5.7
TF16	70	20	10
TF17	70	24	6
TF18	70	25.7	4.3

Construction of ternary phase diagrams

Ternary phase diagrams of the selected oils, surfactants and co-surfactants at various proportions were constructed to identify the self-emulsification regions. All the formulations were investigated with various proportions of oil, surfactant and co-surfactant for each system. All the formulations were observed visually immediately for spontaneity of emulsification, clarity, phase separation and precipitation of drug and excipients [21]. Briefly, 0.5 ml formulations were added drop by drop to 500 ml enzyme-free simulated gastric fluid (SGF pH 1.2) at 37.0±0.5 °C; the mixtures were gently stirred on a magnetic stirrer at 100 rpm to simulate the gastrointestinal wriggle. The resultant emulsions were stored for 48 h at ambient temperature and observed for clarity, coalescence of droplets, phase separation and drug precipitation. Emulsions showing phase separation, cracking and coalescence of oil droplets were judged as unstable emulsions. All the studies were repeated three times with and without drug with similar observations made between repeats. The mixtures were considered well dispersed when the formulation spread quickly in SGF and was clear or milk-white color with no phase separation or coalescence after the stirring stopped. Phase diagram was constructed identifying the self-emulsifying region using ProSim Ternary Diagram software.

Characterization of SEDDS

Visual assessment of self-emulsification time and appearance

Assessment of the self-emulsifying properties of SEDDS formulations was performed by visual observation. The USP type II dissolution apparatus (Electrolab, Mumbai, India) was used to assess the efficiency of self-emulsification of different formulations. 1g of each formulation was added dropwise into 500 ml of distilled water maintained at 37 °C with gentle agitation condition provided by rotating paddle at 50 rpm. Begin timing after the formulation was added completely and stop until the homogeneous emulsion was formulated. The appearance of emulsions was monitored and categorized as clear, translucent and cloudy. The *in vitro* performances of the formulations were visually assessed using the grading system as discussed by Khoo *et al.* [22].

Droplet size and zeta (ζ)–potential measurements

The mean droplet size (z average), polydispersity index (PDI) and ζ-potential of stable formulations were determined at 25 °C with a Zetasizer Nano-ZS dynamic light scattering apparatus (Malvern Instruments, UK). Each formulation was diluted with filtered (0.45 µm, Millipore) double distilled water before analysis. Size analysis was carried at 25 °C with an angle of detection of 90 °.

Effect of pH and robustness to dilution

Formulations were subjected to 50, 100, 1000 and 3000 fold dilution with enzyme-free SGF (pH 1.2), enzyme-free simulated intestinal fluid (SIF, pH 6.8) and distilled water. The resultant diluted emulsions were observed for any physical changes such as (coalescence of droplets, phase separation or precipitation of drugs) after 24 h storage [23].

Formulation stability

Selected IRB-loaded formulations underwent three consecutive cooling and heating cycle to assess their stability [24]. Each cycle consisted of cooling the formulation at 4 °C for 24 h in the refrigerator, followed by heating at 45 °C for 48 h in an incubator. The droplet size, PDI, and ζ-potential of the emulsions were determined after each cycle, and moreover every month on formulations stored at 4 °C and 25 °C for up to three months.

Cloud point measurement

The cloud point measurement was carried out for the stable formulations. The formulation was diluted 100 times with distilled water and kept in a water bath which was maintained at a temperature of 25 °C with a gradual increase of temperature at a rate of 5 °C/min and the corresponding cloud point temperatures were read at first sign of turbidity by visual observation [25].

***In vitro* drug release studies**

Drug release experiments were conducted using a modified dialysis method [26]. Initially, the dialysis membrane tubing was soaked in the release medium for 12 h at room temperature which was treated at 40 °C before the start of the experiment. The diluted SMEDDS formulation (equivalent to 20 mg IRB) and 1 ml IRB suspension (20 mg IRB in SGF pH 1.2 as the control) were placed in dialysis tubing and clamped on both sides. The secured dialysis tube was tied to the paddle of the apparatus and allowed to rotate freely in the dissolution vessel of USP type-II dissolution apparatus (Electrolab Dissolution Tester (USP) TDT-06L, Mumbai, India) containing 500 ml of enzyme-free SGF (pH 1.2) at 37±0.5 °C and stirred at 50 rpm. An aliquot of 5 ml was withdrawn at predetermined time intervals (15, 30, 45, 60, 75, 90, 120, 150 180 and 240 min) and filtered through 0.45 µ filter. The withdrawn volume was replenished immediately with the same volume of fresh medium in order to keep total volume constant and maintain sink conditions. The concentration of IRB in the filtrate was analyzed using UV spectrophotometer at 294 nm. The blank SEDDS without drug was carried out similarly and used as a reference to circumvent interference from the formulation components if any. The mean of at least three determinations was used to calculate the drug release.

Pharmacokinetic studies

In vivo studies were carried out as per the guidelines of the Institutional Animal Ethics Committee (Regd. No. IAEC/GIP-1287/SKS-F/Approved/10/2017-18). New Zealand white rabbits (1.8–2.0 kg) of either sex were housed under standard conditions of temperature, relative humidity, and light. Unless otherwise specified, food and water were given *ad libitum*.

All animals were separated into two groups [Group-I and Group-II], with 6 animals in each group and fasted for 24 h. The IRB (1 mg/kg) pure drug in aqueous suspension in 0.5% sodium carboxymethyl cellulose for Group-I animals and optimized SMEDDS formulation (equivalent to 1 mg/kg IRB) for Group-II animals were administered orally with the help of oral feeding needle. Water was given *ad libitum* during fasting and throughout the experiment.

After drug administration, 1 ml of blood sample was collected from marginal ear vein at time intervals of 0, 0.5, 1, 1.5, 2, 3, 4 and 6 h in the precoated EDTA tubes. The samples were centrifuged at 3000 rpm for 15 min and the separated plasma samples were stored at refrigerated conditions (2–8 °C) until analysis. Irbesartan contents of the plasma samples were determined by HPLC method.

Estimation of pharmacokinetic parameters

The pharmacokinetic parameters for the drug in control and optimized SEDD formulation following oral administration were determined from plasma concentration data. Various pharmacokinetic parameters such as peak plasma concentration (C_{max}), time of peak plasma concentration (t_{max}), the area under the curve (AUC) were calculated in each case using the data. The total area under the concentration-time curve (AUC) from time zero to 8 h were be calculated by the trapezoidal rule method. The maximal concentration (C_{max}) and the time to maximal concentration (t_{max}) were obtained directly by observation. The relative bioavailability (BA) of SMEDDS form to the control was calculated using the following equation.

$$\% \text{ Relative B.A.} = \frac{AUC_{test}}{AUC_{Reference}} \times \frac{Dose_{reference}}{Dose_{test}} \times 100$$

The pharmacokinetic parameters were performed by non-compartmental analysis. All values are expressed as the mean±SD

Statistical treatment of the data

The pharmacokinetic data of TF9 SMEDDS and reference formulations were compared by the Student's t-test. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Solubility studies

The drug-loading capacity of the SEDDS formulations depends on the solubility of IRB in the various vehicles of the system, which was determined by solubility studies. The results are presented in fig. 1.

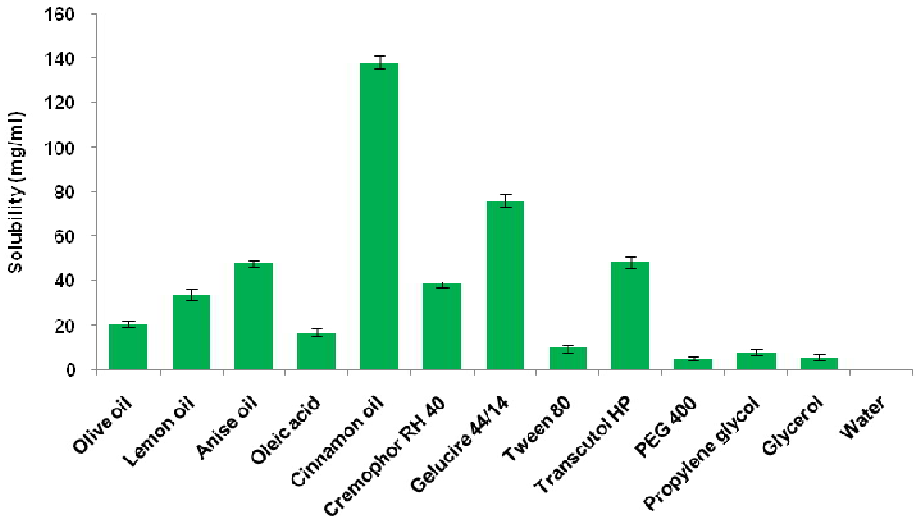

Fig. 1: Solubility of Irbesartan in various vehicles; each value is expressed as mean±SD (n = 3)

FTIR study

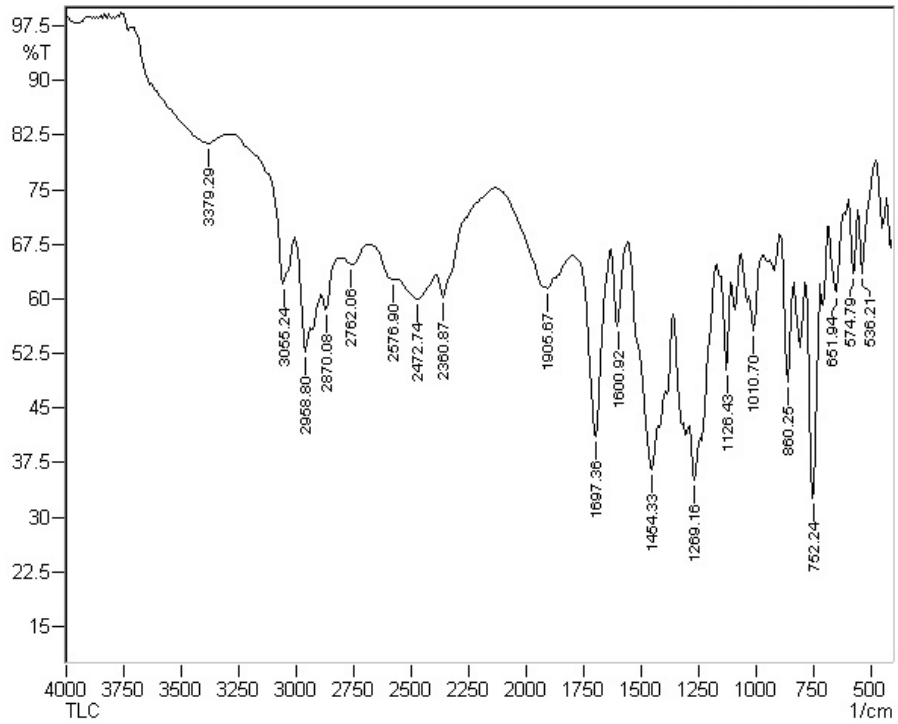

Fig. 2: FTIR spectrum of Irbesartan

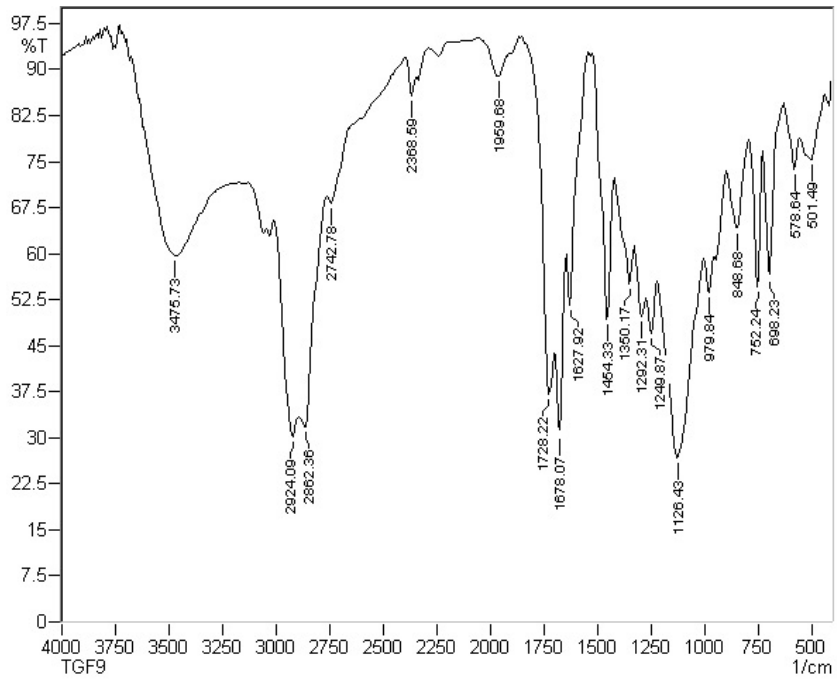

Fig. 3: FTIR spectrum of SEDDS (TF9)

Ternary phase diagram

A ternary phase diagram was investigated for the prepared formulations. Before the construction of ternary phase diagrams, the miscibility between surfactants and oils was investigated to select the best components. The mixture of surfactant Gelucire 44/14 and cinnamon essential oil resulted in clear solutions. Formation of emulsion systems (the green area in fig. 4) was observed at ambient temperature.

Self-emulsification efficiency and appearance

The efficiency of self-emulsification could be estimated by determining the rate of emulsification. The results of rate of emulsification are shown in table 2. The results suggested that all the formulations except TF7 and TF8, up to 40% oil content showed the emulsification time of less than 60 sec.

Droplet size and zeta (ζ) potential

Droplet size, PDI and ζ-potential of the optimized SEDDS in SGF with (20 mg/ml) and without IRB are listed in table 3.

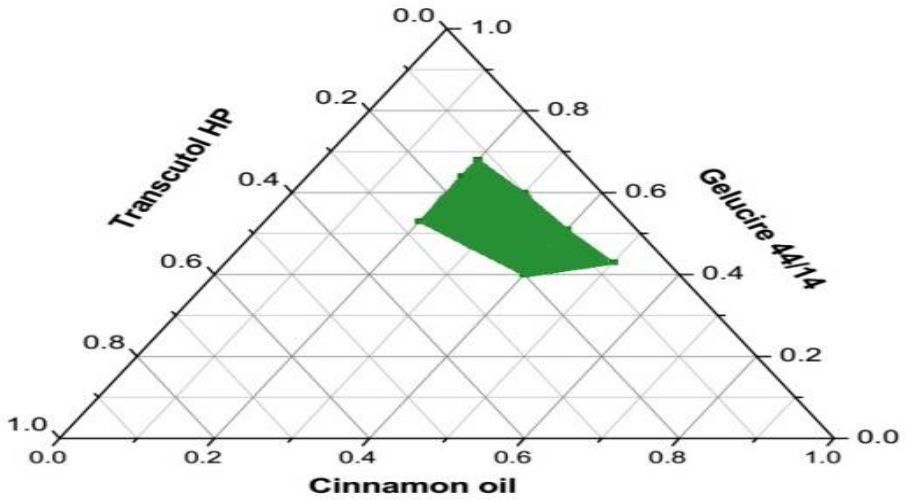

Fig. 4: Ternary phase diagram of SEDDS between cinnamon oil, gelucire 44/14 and transcutol HP (green domain indicates the region of self emulsification

Table 2: Evaluation parameters of Irbesartan loaded SEDDS formulations

Formulation	Self-emulsification time* [Sec]	Clarity*	Stability after 48 h*	Visual grading*
TF1	32±3	Translucent	Unstable	B
TF2	24±1	Transparent	Stable	A
TF3	21±2	Transparent	Stable	A
TF4	42±4	Translucent	Unstable	C
TF5	34±2	Translucent	Stable	B
TF6	30±3	Transparent	Stable	A
TF7	103±4	Cloudy	Unstable	C
TF8	82±3	Translucent	Stable	B
TF9	54±2	Translucent	Stable	B
TF10	142±4	Cloudy	Unstable	D
TF11	119±3	Cloudy	Unstable	C
TF12	112±2	Translucent	Unstable	C
TF13	171±4	Cloudy	Unstable	D
TF14	138±4	Cloudy	Unstable	D
TF15	125±3	Cloudy	Unstable	C
TF16	208±4	Cloudy	Unstable	D
TF17	184±6	Cloudy	Unstable	D
TF18	162±5	Cloudy	Unstable	D

*Data expressed as mean±SD (n=3)

Table 3: Droplet size, PDI and ζ-potential of optimized SMEDDS in 500 ml SGF (pH 1.2) at room temperature, with and without drug

Formulation	Without drug			With drug (20 mg/ml)		
	Droplet size* [nm]	PDI	Zeta potential* [mV]	Droplet size* [nm]	PDI	Zeta potential* [mV]
TF2	201.98±9.24	0.192	-7.39±2.61	236.58±10.37	0.205	-7.53±2.91
TF3	187.65±8.18	0.201	-7.25±2.86	205.26±9.61	0.227	-7.59±2.72
TF5	198.54±6.77	0.192	-5.53±2.29	213.82±5.96	0.186	-6.04±2.15
TF6	135.52±7.31	0.283	-8.26±2.41	161.14±8.48	0.276	-8.37±2.84
TF8	179.37±9.46	0.289	-7.01±2.35	197.65±7.29	0.315	-6.57±2.38
TF9	134.85±3.55	0.275	-4.52±0.72	150.72±3.64	0.290	-4.58±0.96
TF12	262.46±7.39	0.316	-4. 95±1.52	278.37±6.18	0.328	-5.75±1.43

*Data expressed as mean±SD (n=3)

Fig. 5: Droplet size distribution of Irbesartan-loaded microemulsion (TF9)

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -4.58	Peak 1:	-4.58	100.0	0.96
Zeta Deviation (mV): 0.96	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.200	Peak 3:	0.00	0.0	0.00
Result quality : Good				

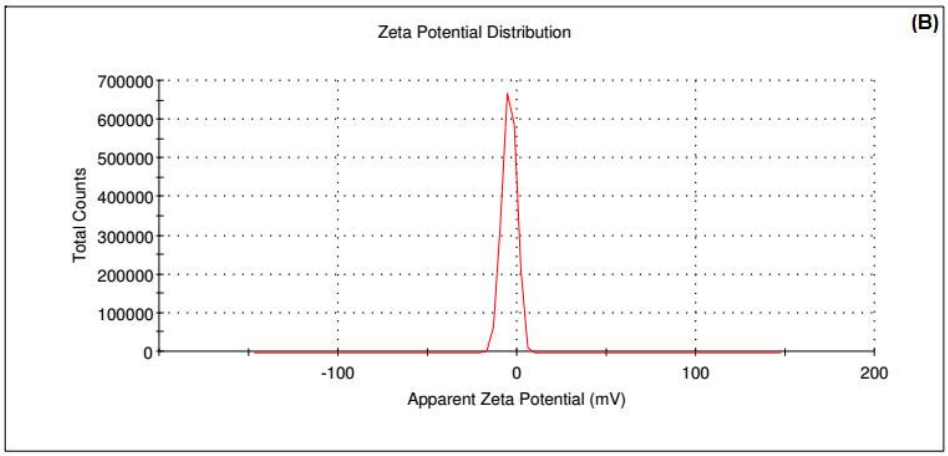

Fig. 6: Zeta potential of Irbesartan-loaded microemulsion (TF9)

Effect of pH and robustness to dilution

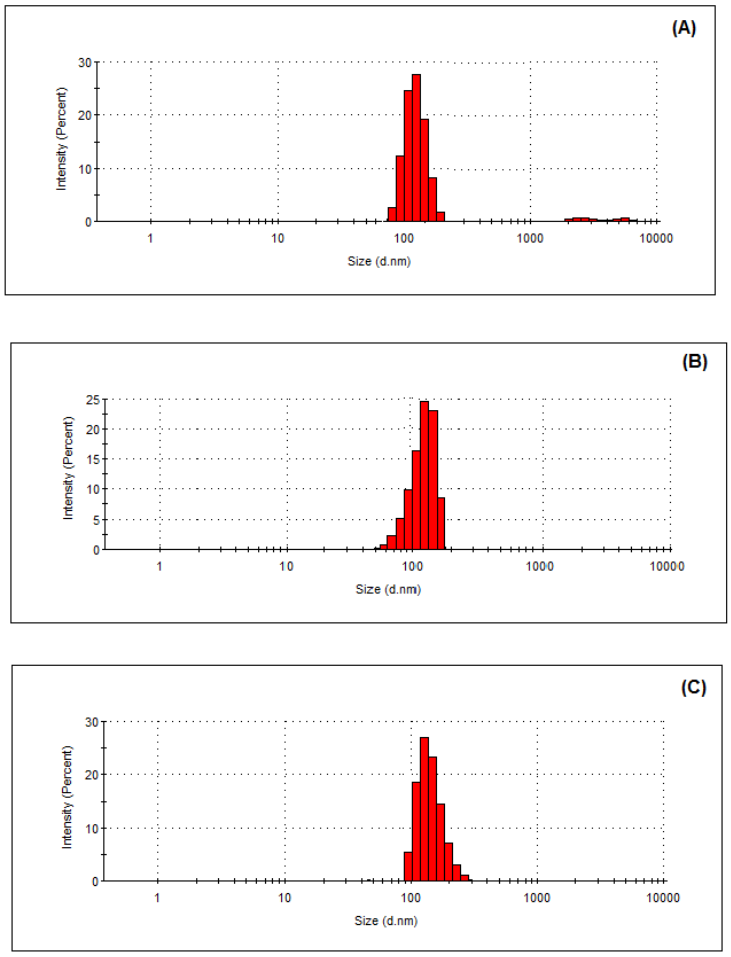

Fig. 7: Particle size distributions of the micro emulsions generated from Formulation TF9 in (A): enzyme free simulated gastric fluid (pH 1.2), (B): enzyme free simulated intestinal fluid (pH 6.8) and (C): distilled water

Table 4: Effects of cooling and heating cycles on the dynamic characteristics of micro emulsions obtained from TF9 containing 20 mg/ml Irbesartan in enzyme free SGF (pH 1.2, 500 ml)

Cooling (4 °C) and heating (45 °C) cycle	Droplet size* [nm]	PDI	Zeta potential* [mV]
--	150.72±3.64	0.290	-4.82±0.96
First	154.68±2.85	0.293	-4.81±0.85
Second	155.29±4.38	0.305	-4.79±0.99
Third	157.16±3.47	0.287	-4.83±0.91

*Data expressed as mean±SD (n=3)

Table 5: Effects of storage conditions on the dynamic characteristics of microemulsion obtained from TF9 containing 20 mg/ml Irbesartan in enzyme free SGF (pH 1.2, 500 ml)

Storage Time (months)	Temperature, 4 °C		Temperature, 25 °C	
	Droplet size* [nm]	PDI	Droplet size* [nm]	PDI
1	150.36±5.86	0.214	151.45±4.39	0.383
2	150.87±6.69	0.371	152.92±7.72	0.412
3	151.12±5.25	0.358	153.36±7.45	0.425

*Data expressed as mean±SD (n=3)

Formulation stability

The stability of TF9, after three cooling and heating cycles, is summarized in table 4 and the effect of storage conditions on the microemulsion stability is presented in table 5.

Cloud point measurement

The cloud point is the temperature above which the clarity of formulation turns to cloudiness. This is due to drug precipitation and phase separation of the emulsion. Since both the drug solubilization and stability of emulsion decreases with phase separation, cloud point should be preferably above 37 °C.

The cloud point temperatures of different formulations determined were in the range of 62–76 °C.

***In vitro* drug release study**

To facilitate the real drug release pattern the dialysis bag method was utilized in drug release studies and the drug release pattern of SMEDDS shown in fig. 8.

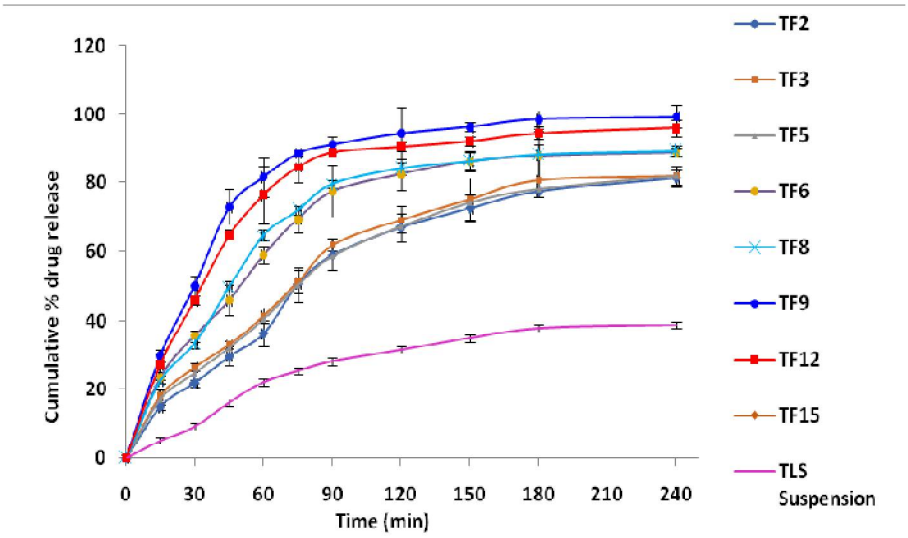

Fig. 8: *In vitro* release profile of Irbesartan suspension and Irbesartan-SMEDDS (Emulsified with SGF, pH = 1.2, 10 ml) in SGF (pH = 1.2, 500 ml). Data expressed as mean±SD (n=6)

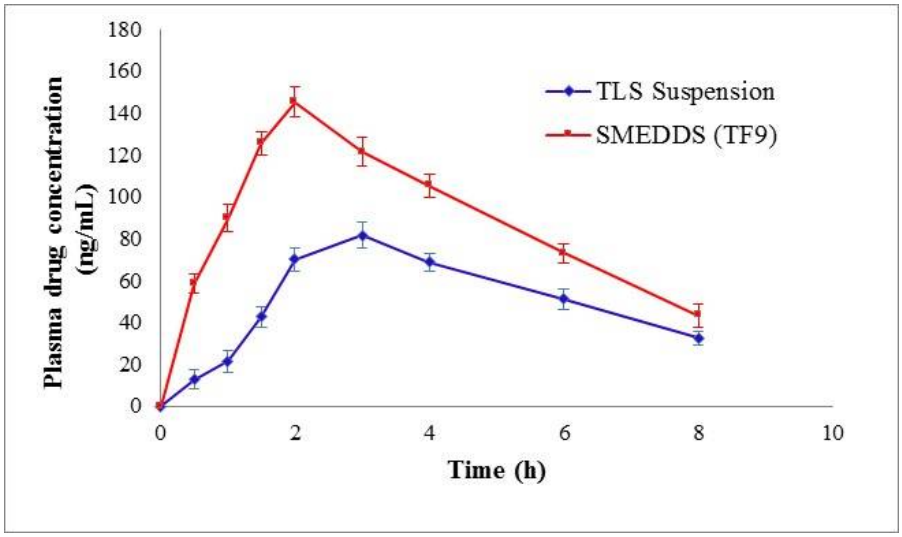

Fig. 9: Plasma concentration of Irbesartan after oral administration of IRB suspension and SMEDDS (TF9) to rabbits. Data expressed as mean±SD (n=6)

Table 6: Pharmacokinetic parameters of Irbesartan after oral administration of IRB suspension and SMEDDS (TF9) in rabbits

Parameters*	IRB suspension	SMEDDS
C_{max} (µg/ml)	81.62±5.99	145.28±7.34
t_{max} (h)	3.00±0.00	2.00±0.00
$AUC_{(0-8)h}$ (µg h/ml)	425.48±11.35	1013.21±15.42
Relative bioavailability (%)	--	238.15

Each value expressed as mean±SD (n = 6)., *P<0.05 by the Student t-test.

Pharmacokinetic studies

The plasma concentration versus time profiles of IRB in rabbits for SMEDDS and IRB suspension following oral administration are presented in fig. 9. The pharmacokinetic parameters of IRB were computed and tabulated in table 6.

DISCUSSION

Among the five oils those have been tested, IRB is highly soluble in cinnamon essential oil (about 138.09±2.82 mg/ml) which is better than anise oil (47.92±1.32 mg/ml), lemon essential oil (34±2.66 mg/ml), olive oil (20.59±1.09 mg/ml) and oleic acid (16.78±1.78 mg/ml). The surfactant has a pivotal role in stabilizing microemulsions, its nature and amount determining droplet size and stability [27]. Nonionic surfactants are usually preferred because of their lower toxicity and higher stability to the effect of pH and ionic strength than ionic and amphiphilic surfactants [28]. The hydrophilic-lipophilic balance (HLB) is a measure of the degree to which a substance is hydrophilic or lipophilic [29]. An HLB value of 20 defines a fully hydrophilic molecule, while a value of 0 defines a lipophilic one [30]. The stability of emulsions depends also on the ratio between the high HLB and low HLB surfactant amounts [21,31]. As shown in fig. 1, among all the investigated surfactants, IRB exhibited quite higher solubility in Gelucire 44/14 (HLB 14) 76.02±2.79 mg/ml than Cremophor RH40 (HLB 15), 38.5±1.45 mg/ml; Tween 80 (HLB 15), 9.75±1.68 mg/ml; and the former was selected for further investigations. The solubility of Irbesartan in different co-surfactants was investigated and a higher solubility was found in Transcutol HP (48.53±2.75 mg/ml) than propylene glycol (8.03±1.45 mg/ml), polyethylene glycol 400 (5.32±1.04 mg/ml) and glycerol (5.79±1.72 mg/ml). So Transcutol HP was selected as co-surfactant which helps in further lowering of interfacial tension. Based on the solubility results the SEDDS formulations were developed employing varying concentrations of cinnamon oil (20–70%), Gelucire 44/14 (20–69%), and Transcutol HP (4–27%).

FTIR spectra of Irbesartan (fig. 2) showed characteristic peaks of aliphatic C-H stretching at 2958 cm^{-1}, C=O stretching at 1697 cm^{-1}, C-N stretching at 1126 cm^{-1}, C=N stretching at 1600 cm^{-1}. The FTIR spectra of SEDDS (fig. 3) also showed all these characteristic peaks with minor shifts. These results from FTIR spectral analysis indicated that there was no chemical interaction between drug and excipients used in the formulation.

Ternary phase behavior investigations help to choose the proper concentration of excipients i.e. oil proportion and optimum S/CoS ratio in the formulation to produce emulsions with good stability [32]. As a fact, all surfactants are potentially irritant or are poorly tolerated [33], so large amounts of surfactants may cause irritation in the gastrointestinal tract [34]; systems which contain a higher proportion of essential oil should be preferred. Since the free energy required to form an emulsion is very low, due to the surfactant which reduces the interfacial tension, the formation is thermodynamically spontaneous. Surfactants also provide a mechanical barrier to coalescence [35]. After observing clarity, stability after 48 h, it was noted that all formulations with S/CoS ratio of 6:1 except TF15 and TF18 i.e. TF3, TF6, TF9 and TF12 produced stable emulsions, whereas the resultant emulsions of formulations with S/CoS ratio of 2:1 showed phase separation and precipitation (results are shown in table 2). Among the S/CoS ratio of 4:1, formulations TF2, TF5, and TF8 also produced stable emulsions. The reason for this may be due to the water solubility of Transcutol HP and its tendency to redistribute between aqueous phase and emulsion–water interface, leading to loss of solvent capacity resulting in an unstable emulsion.

However, with an increase of oil proportion over 40% to 70%, the emulsification time was increased up to more than 200 sec. These visual observations indicated that higher the proportion of surfactant system, greater the spontaneity of emulsification. This may be due to excessive penetration of aqueous phase into the oil phase causing very large interfacial disruption and expulsion of droplets into the bulk aqueous phase [36]. SEDDS that passed this test in grades A and B were selected for further evaluation, as grades A and B formulations will remain as SNEDDS or SMEDDS when dispersed in G. I. fluids. All other SEDDS that were falling in grades C, D and E were discarded for further evaluation.

In agreement with P. Patil *et al.* (2007) [37], a slight increase in droplet size was observed for the IRB-loaded SEDDS. This can be attributed to the preferential dissolution of the drug in the interfacial film (formed by the surfactant and co-surfactant) that increases the interfacial tension. Moreover, the addition of the drug could induce surfactant aggregation, thus reducing its efficiency. The PDI values were below 0.5 indicated that the droplets were uniform in size. The ζ-potential is correlated to the electrostatic repulsion and aggregation of the droplets. High positive or negative ζ-potential values (higher electrostatic repulsive forces) arrest coalescence, thus enabling stability of the emulsions [38-40]. The negative charges were due to the presence of free fatty acids in the surfactant [19, 28]. The droplet sizes of all the optimized formulations except TF12 were below 250 nm suggested SMEDD formulations. Again among SMEDDS, the formulation TF9 incorporate high proportions of oil as compared to conventional SMEDD formulations where the later is prepared by incorporating a large amount of surfactant or surfactant-cosurfactant mixture (>70%). The droplet size of TF9 with Irbesartan was found to be 150.72±3.64 nm (fig. 5) with PDI of 0.290. The zeta potential of the emulsion developed by TF9 was found to be -4.58±0.96 mV (fig. 6). The conductivity of the emulsion was 0.200 mS/cm, which means the emulsion was fine oil in water (conductivity>10 µS/cm) [27].

The stable SMEDDS formulations exposed to different pH media such as enzyme-free SGF (pH 1.2), enzyme-free SIF (pH 6.8) and distilled water to mimic the *in vivo* conditions revealed no precipitation or phase separation indicating all the formulations were found to be robust towards different pH conditions (fig. 7). However, the formulations were robust over the wider degree of dilution without any signs of drug precipitation and phase separation.

The droplet size increased with no significant changes of the ζ-potential after three cooling and heating cycles. Moreover, the formulation didn't exhibit any drug precipitation or phase separation during the whole process. No marked difference of droplet size was observed for formulations stored at 4 °C or 25 °C (table 5). The above findings indicated that this Irbesartan loaded formulation is thermodynamically stable.

The reason for higher cloud point temperature may be attributed to the solubility of the drug in oil and surfactant system, the optimized ratio of S/CoS and/or surfactants with higher HLB values. This infers good thermal stability of all the tested formulations. Above 76 °C, phase separation and precipitation was observed, this is due to dehydration of surfactant mixture [23].

The drug release from SMEDDS was significantly greater than that of the Irbesartan suspension. In 2 h, the SMEDDS TF6, TF8, TF9 and TF12 released more than 80% of the drug in comparison to 31.5% of the Irbesartan from suspension. The formulations TF9 and TF12 released almost all drug (>95%) in compared to other SMEDDS, with just a small difference among the different SMEDDS that are consistent with the droplet sizes. In addition, the release from SMEDDS was faster, further supporting the hypothesis that micro-scale emulsions can improve the release of lipophilic drugs. The drug release pattern of SMEDDS reveals that the highest drug release was observed with TF9 formulation after 60 min. that could be due to proper compromise between proportions of oil and surfactant in the system. Though the formulations TF2, TF3 and TF6 produced emulsions with better spontaneity and more clarity, these formulations showed 36.29±3.91%, 41.61±2.17% and 59.26±2.62% drug release respectively, this may

be due to high surfactant proportion in the formulation. However, the high surfactant proportion is usually concomitant with a higher probability of surfactant migration into surrounding aqueous media upon dispersion which is supposed to form micelles that trap free drug inside, with subsequent hindrance in drug release [33]. The drug release pattern from TF9 formulation followed first order up to 1 h.
Results of the pharmacokinetic study showed that the C_{max} and $AUC_{(0-8\ h)}$ of IRB in SMEDDS increased by 1.78-fold and 2.38 fold respectively compared to the IRB suspension. Additionally, the IRB in SMEDDS was absorbed more rapidly and reached its peak concentration faster ($p<0.05$). The absorption of Irbesartan from SMEDDS resulted in a 2.4-fold increase in bioavailability compared with the suspension formulation.

CONCLUSION

In the present study, a novel SMEDDS was successfully designed as a stable, high essential oil ratio (40%) and high drug-loaded (approximate 20%) formulation for the solubility and dissolution rate enhancement of practically water insoluble drug, Irbesartan. The formulation composition and pH of the emulsifying medium significantly impacted the droplet size. The stability study confirmed that the SMEDDS formulations could withstand various storage conditions with excellent stability. The *in vitro* drug release study demonstrated that the release from SMEDDS was more efficient when compared with the drug suspension. Also *in vivo* studies for clinical purpose, SMEDDS showed significantly greater extent of absorption than the suspension formulation. The relative bioavailability of SMEDDS to the suspension formulation (20 mg/ml) was 238%. Under these circumstances, the present SMEDDS would be a promising novel system to improve the aqueous solubility of poorly soluble drug and potentially the bioavailability.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Authors declared that there is no conflict of interest

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