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Research paper

Development and bioavailability assessment of ramipril nanoemulsion formulation

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Abstract

The objective of our investigation was to design a thermodynamically stable and dilutable nanoemulsion formulation of Ramipril, with minimum surfactant concentration that could improve its solubility, stability and oral bioavailability. Formulations were taken from the o/w nanoemulsion region of phase diagrams, which were subjected to thermodynamic stability and dispersibility tests. The composition of optimized formulation was Sefsol 218 (20% w/w), Tween 80 (18% w/w), Carbitol (18% w/w) and standard buffer solution pH 5 (44% w/w) as oil, surfactant, cosurfactant and aqueous phase, respectively, containing 5 mg of ramipril showing drug release (95%), droplet size (80.9 nm), polydispersity (0.271), viscosity (10.68 cP), and infinite dilution capability. *In vitro* drug release of the nanoemulsion formulations was highly significant ($p < 0.01$) as compared to marketed capsule formulation and drug suspension. The relative bioavailability of ramipril nanoemulsion to that of conventional capsule form was found to be 229.62% whereas to that of drug suspension was 539.49%. The present study revealed that ramipril nanoemulsion could be used as a liquid formulation for pediatric and geriatric patients and can be formulated as self-nanoemulsifying drug delivery system (SNEDDS) as a unit dosage form.
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Keywords: Nanoemulsion; Ramipril; Bioavailability; SNEDDS; Phase diagrams; Solubility; Sefsol 218

1. Introduction

Poor bioavailability can be due to poor solubility, degradation in GI lumen, poor membrane permeation and presystemic elimination [1,2]. By many estimates up to 40 percent of new chemical entities (NCEs) discovered by the pharmaceutical industry today and many existing drugs are poorly soluble or lipophilic compounds which leads to poor oral bioavailability, high intra- and inter-subject variability and lack of dose proportionality [3]. Thus, for such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution [4]. The ability to deliver poorly soluble drugs will grow in significance in the coming years as innovator companies rely upon NCEs for a larger share of the revenue within the pharmaceutical market.

In recent years, much attention has focused on lipid-based formulations to improve the oral bioavailability of poorly water soluble drug compounds. In fact, the most popular approach is the incorporation of the active lipophilic component into inert lipid vehicles such as oils, surfactant dispersions, microemulsions, nanoemulsions, self-emulsifying formulations, self-microemulsifying formulations, emulsions and liposomes [5–19]. Most of them increase surface area of the drugs to improve solubilisation behaviour, as well as permeation. From the viewpoint of oral drug delivery, lipids are studied as components of various oily liquids and dispersions that are designed to increase solubility and bioavailability of

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drugs belonging to the class II and IV of the biopharmaceutical drug classification system [4].

One of the promising technologies is nanoemulsion drug delivery system, which is being applied to enhance the oral bioavailability of the poorly soluble drugs. Nanoemulsions are thermodynamically stable, transparent (or translucent); dispersions of oil and water stabilized by an interfacial film of surfactant molecules having the droplet size less than 100 nm. Nanoemulsion provides ultra low interfacial tensions and large o/w interfacial areas. Nanoemulsions have a higher solubilization capacity than simple micellar solutions and their thermodynamic stability offers advantages over unstable dispersions, such as emulsions and suspensions, because they can be manufactured with little energy input (heat or mixing) and have a long shelf life. The nanosized droplets leading to enormous interfacial areas associated with nanoemulsions would influence the transport properties of the drug, an important factor in sustained and targeted drug delivery [20,21]. The attraction of formulating o/w nanoemulsion systems lies in their ability to incorporate hydrophobic drugs into the oil phase thereby enhancing their solubility [21]. Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible [3,21–24].

Ramipril {(2*S*,3a*S*,6a*S*)-1-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl] amino]-1-oxopropyl]octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid}, a potent antihypertensive drug, is almost completely converted to its active metabolite ramiprilat (a dicarboxylic acid) by hydrolytic cleavage of the ester group in the liver which has about six times angiotensin converting enzyme (ACE) inhibitor activity of ramipril. Ramipril is a highly lipophilic (log *P* (octanol/water), 3.32), poorly water soluble drug with absolute bioavailability of 28–35%, when 5 mg of oral ramipril is compared with the same dose given intravenously [25–27]. Ramipril and ramiprilat inhibit ACE which catalyses the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Inhibition of ACE receptor decreases tissue and circulating ACE activity, which leads to decreased vasopressor activity and decreased aldosterone secretion and therefore, causes general vasodilatation and lowers blood pressure effectively [28,29]. The HOPE (heart outcomes prevention evaluation) trial showed that ramipril therapy significantly reduced the incidence of myocardial infarction, stroke or cardiovascular death (relative risk 0.78) and reduced total mortality (relative risk 0.84) versus placebo in patients (mean age of patients was 65.9 years) with atherosclerotic diseases or diabetes mellitus [30]. According to study conducted by ESCAPE trial group, ramipril is a very effective antihypertensive and antiproteinuric agent in children with chronic renal failure associated with hypertension [31].

The dose of ramipril varies between 2.5 mg and 20 mg and frequently prescribed dose is 5 mg for the adult. Therefore, for the present study, 5 mg dose was selected for the development of nanoemulsion formulation. Thus, the objectives of the present study were to develop and characterize an optimal nanoemulsion formulation of ramipril using minimum surfactant concentration, so that nano-sized droplets could be maintained on dilution by the gastrointestinal (GI) fluids with an aim to increase its bioavailability and compare it with a marketed capsule formulation as well as the drug suspension. The other objective was to develop a liquid formulation that can be used for pediatric patients as well as to develop a self-nanoemulsifying drug delivery (SNEDDS) unit dose formulation.

2. Materials and methods

2.1. Materials for component selection

Ramipril base was a gift sample from Ranbaxy Research Laboratories (Haryana, India). Medium chain triglyceride (Labrafac®), Caprylo caproyl macrogol-8-glyceride (Labrasol®), Polyglyceryl-6-dioleate (Plurol oleique®) were gift samples from Gattefossé (Saint Priest, Cedex France), Propylene glycol mono caprylic ester (Safsol 218®) was gift sample from Nikko Chemicals (Tokyo, Japan). Isopropyl myristate (IPM), Glycerol triacetate (Triacetin), Castor oil, Polyoxyethylene (20) sorbitan mono oleic acid (Tween 80®), Diethylene glycol monoethyl ether (Carbitol®), Sodium perchlorate AG, and Acetonitrile (HPLC grade) were purchased from Merck (Schuchardh, Hokenbrunn, Germany). Water was obtained from Milli-Q-water purification system (Millipore, MA). For LC/MS/MS study, all the chemicals and C-18 solid phase extraction cartridges (Oasis HLB, 30 ng/cc) were a gift from Ranbaxy Research Lab. Ltd. (Haryana, India). All other chemicals were of analytical grade.

2.2. Screening of components

The most important criterion for the screening of components for nanoemulsion is the solubility of poorly soluble drug in oils, surfactants and cosurfactants. Since the aim of this study is to develop an oral formulation, therefore, solubility of drug in oils is more important as the ability of nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in oil phase. The solubility of ramipril in various oils was determined by adding an excess amount of drug in 2 mL of selected oils (Sefsol 218, Triacetin, IPM, Labrafac, Castor oil), distilled water separately in 5 mL capacity stopper vials, and mixed using a vortex mixer. The mixture vials were then kept at 25 ± 1.0 °C in an isothermal shaker (Nirmal International, Delhi, India) for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 μm membrane filter. The concentration of ramipril was determined in oils and water using HPLC at 210 nm.

2.3. Pseudo-ternary phase diagram study

On the basis of the solubility studies of drug, Sefsol 218 was selected as the oil phase. Tween 80 and Labrasol were used as surfactants and Carbitol and Plurol oleique used as cosurfactants. Standard buffer solution (pH 5) [32] was used as an aqueous phase for the construction of phase diagrams. Oil, surfactants and cosurfactants were grouped in four different combinations for phase studies (Table 1). Surfactant and cosurfactant (Smix) in each group (Table 1) were mixed in different weight ratios (1:0, 0.5:1 (1:2), 1:1, 2:1 (1:0.5), 3:1, 1:3, 4:1). These Smix ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for detailed study of the phase diagrams for formulation of nanoemulsion (Figs. 1–4). For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and Smix, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1 (1:0.1), were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo-ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was done to each weight ratio of oil and Smix and visual observation was carried out for transparent and easily flowable o/w nanoemulsions. The physical state of the nanoemulsion was marked on a pseudo-three-component phase diagram with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (Smix ratio).

2.4. Selection of formulations from phase diagrams

From each phase diagram, constructed, different formulations were selected (Tables 3–5) from nanoemulsion region so that drug could be incorporated into the oil phase on the following bases.

(a) The oil concentration should be such that it solubilizes the drug (single dose) completely depending on the solubility of the drug in the oil. Five milligrams of ramipril will dissolve easily in 0.1 mL (10% of 1 mL) of oil.

Table 1
Oil, surfactants and cosurfactants grouped in different combinations

Group	Oil	Surfactant	Cosurfactant
I	Sefsol 218	Labrasol	Carbitol
II	Sefsol 218	Labrasol	Plurol oleique
III	Sefsol 218	Tween 80	Carbitol
IV	Sefsol 218	Tween 80	Plurol oleique

(b) To check if there was any effect of drug on the phase behaviour and nanoemulsion area of the phase diagram.
(c) The minimum concentration of the Smix used for that amount of oil was taken.
(d) For convenience purposes, 1 mL was selected as the nanoemulsion formulation, so that it can be increased or decreased as per the requirement in the proportions. The beauty of this system is the scale up of the proportions is easy, as the system is thermodynamically stable.

Selected formulations were subjected to different thermodynamic stability and dispersibility tests.

2.4.1. Thermodynamic stability studies

1. Heating cooling cycle: Six cycles between refrigerator temperature (4 °C) and 45 °C with storage at each temperature of not less than 48 h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.
2. Centrifugation: Passed formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.
3. Freeze thaw cycle: Three freeze thaw cycles between −21 °C and +25 °C with storage at each temperature for not less than 48 h was done for the formulations.

Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility test for assessing the efficiency of self-emulsification.

2.4.2. Dispersibility test

The efficiency of self-emulsification of oral nanoemulsion was assessed using a standard USP XXII dissolution apparatus 2 [33,34]. One milliliter of each formulation was added to 500 mL of water at 37 ± 0.5 °C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. The *in vitro* performance of the formulations was visually assessed using the following grading system:

Grade A: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance.
Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance.
Grade C: Fine milky emulsion that formed within 2 min.
Grade D: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).
Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

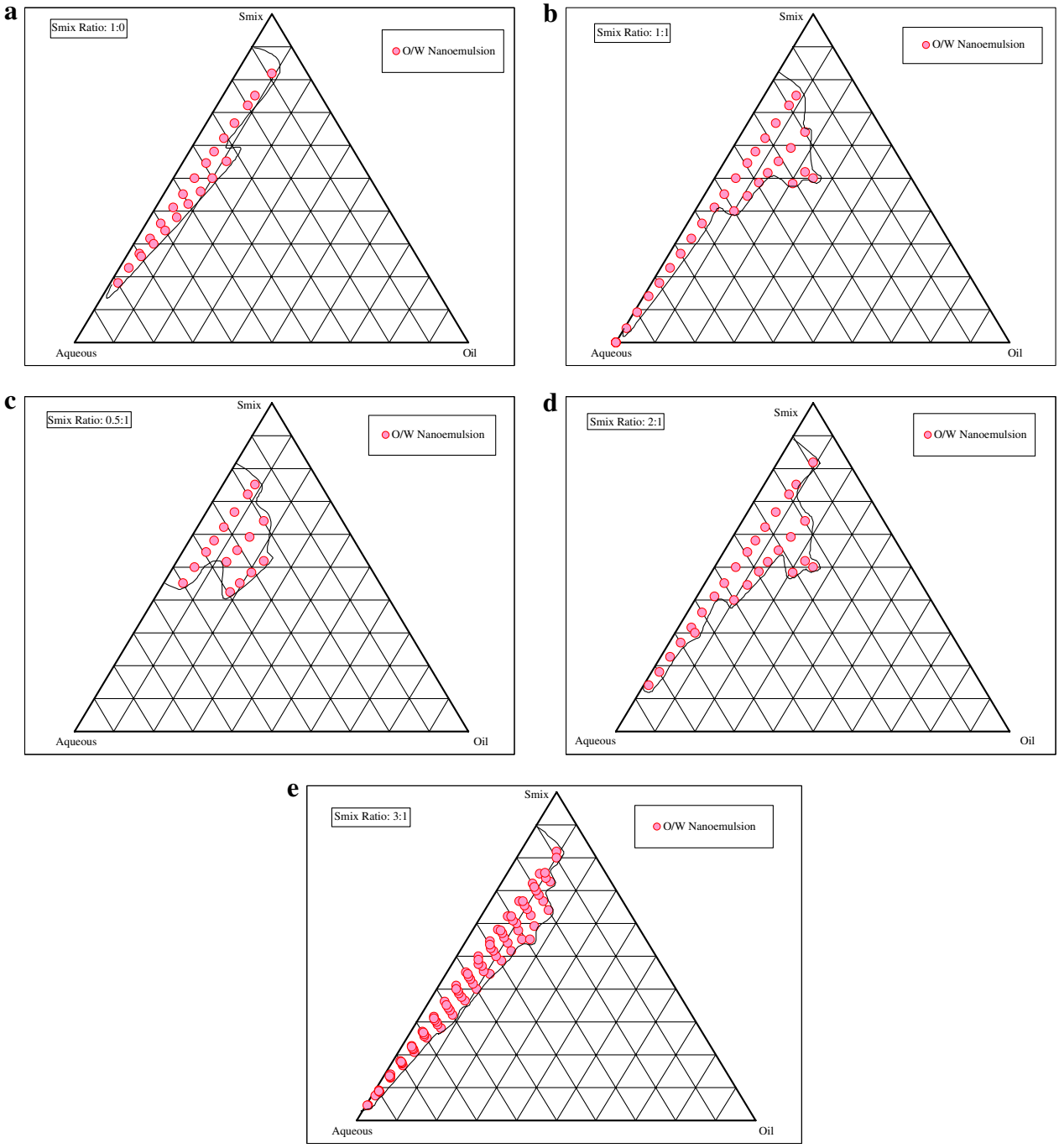

Fig. 1. Pseudo-ternary phase diagrams of Group I, indicating o/w nanoemulsion region at different Smix ratios.

Those formulations that passed the thermodynamic stability and also dispersibility test in Grade A and Grade B were selected for further studies (Table 6). The selected formulations were prepared by dissolving 5 mg (single dose) of ramipril in oil (10%, 15%, 20%, 25%, 30%). Respective Simx ratio was added to the oil, mixed on the vortex mixer and aqueous phase added. The mixture was mixed on the mixer and the resulting mixture gave nanoemulsion. The quantities added are given in Table 6.

2.5. Globule size analysis

The formulation (0.1 mL) was dispersed in 50 mL of water in volumetric flask and gently mixed by inverting the flask. Globule size of the nanoemulsion was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles [35], using a Zetasizer 1000 HS (Malvern Instruments, UK). Light scattering was monitored at 25 °C at a 90° angle (Table 7).

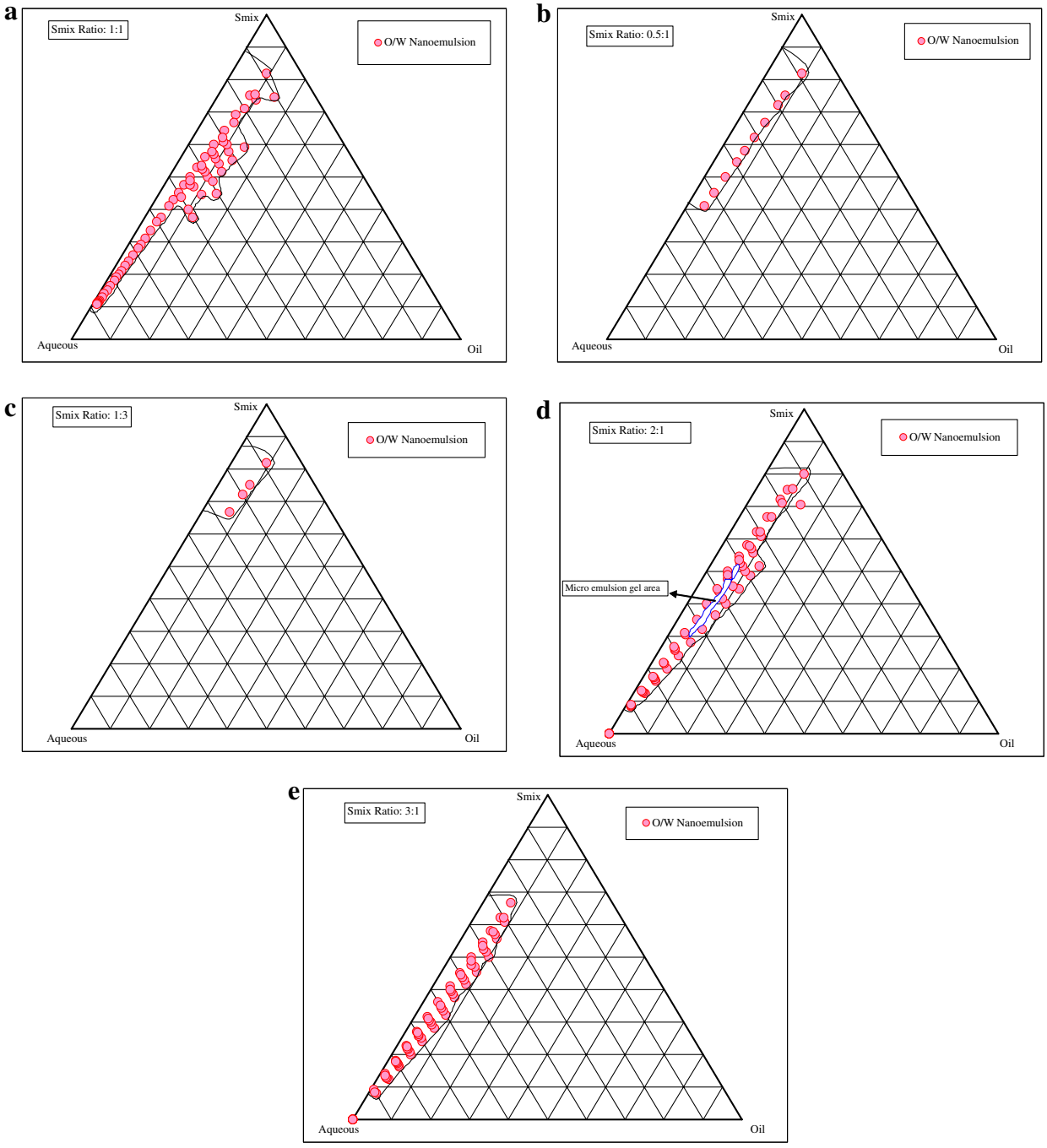

Fig. 2. Pseudo-ternary phase diagrams of Group II, indicating o/w nanoemulsion region at different Smix ratios.

2.6. Viscosity

The viscosity of the formulations (0.5 g) was determined as such without dilution (Table 7) using Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using spindle # CPE40 at 25 ± 0.5 °C. The software used for the calculations was Rheocalc V2.6.

2.7. Transmission electron microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM) TOPCON 002B operating at 200 kV capable of point-to-point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion.

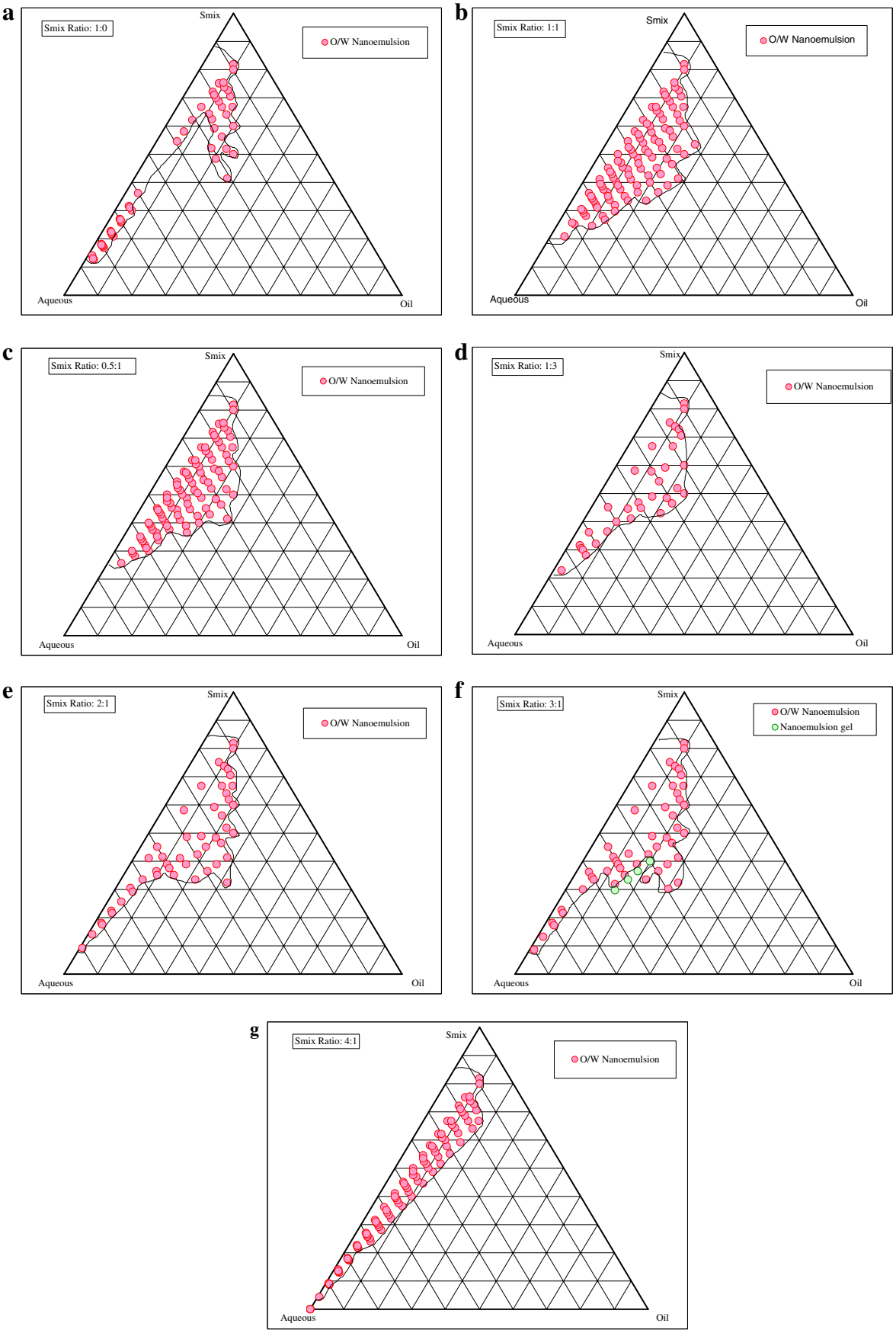

Fig. 3. Pseudo-ternary phase diagrams of Group III, indicating o/w nanoemulsion region at different Smix ratios.

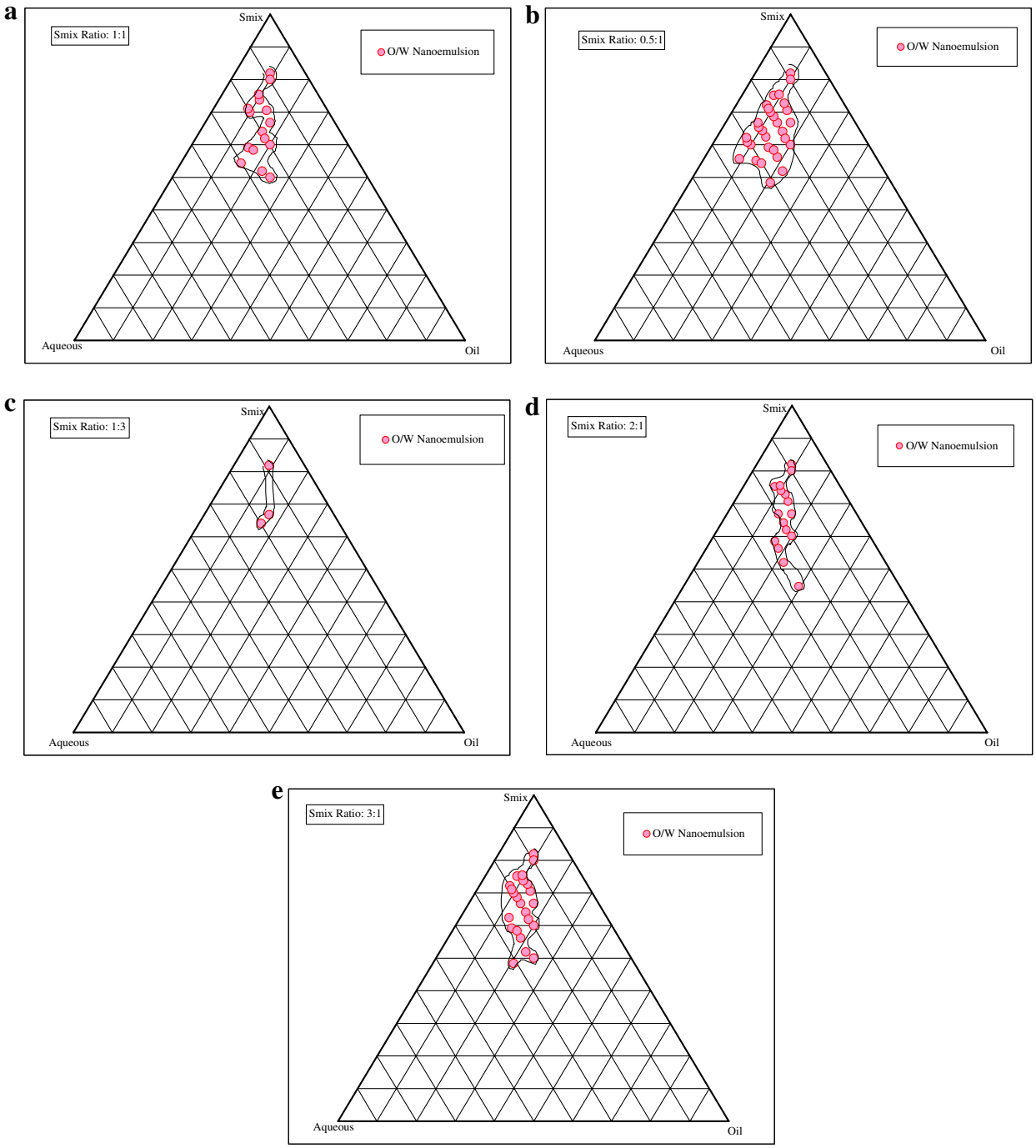

Fig. 4. Pseudo-ternary phase diagrams of Group IV, indicating o/w nanoemulsion region at different Smix ratios.

In order to perform the TEM observations, the nanoemulsion formulation was diluted with water (1/100). A drop of the diluted nanoemulsion was then directly deposited on the holey film grid and observed after drying (Fig. 5).

2.8. *In vitro* drug release

In vitro release test was performed in 500 ml of distilled water, which was based on USP XXIV method (Dissolution apparatus # 2, at 50 rpm and 37 ± 0.5 °C) [18]. One milliliter of nanoemulsion formulation (single dose containing 5 mg of ramipril) was placed in dialysis bag (MWCO 12,000 g/mole; Sigma, USA). Samples (1 mL) were withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 16, 20, 24 h) and aliquot amount of distilled water was replaced. The release of drug from nanoemulsion formulation was compared with the conventional capsule formulation (Corpril® 5) and the suspension of pure drug. The samples were analyzed for the drug content using

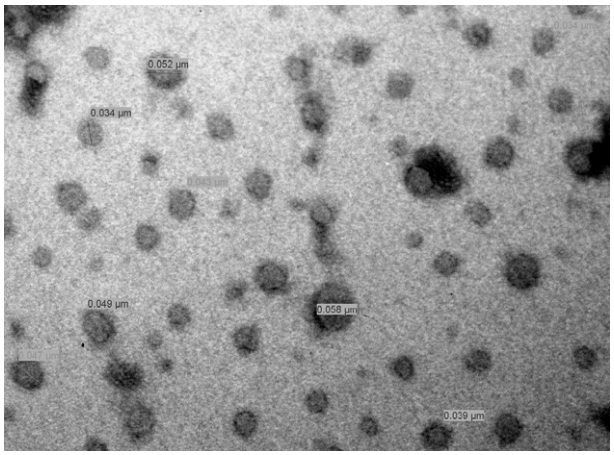

Fig. 5. Transmission electron microscopic positive image of ramipril nanoemulsion showing size of some oil globules.

HPLC method at 210 nm (Fig. 6). In vitro drug release data were analyzed by one-way analysis of variance (ANOVA) using Dunnett's test.

2.9. HPLC analysis of ramipril

The concentration of the ramipril in *in vitro* samples was determined by HPLC method [36]. The system consisted of Shimadzu LC-10A VP with a UV detector (Shimadzu, Japan). The software used in the system was Class VP, version 5.032. The chromatographic column was a RP C_{18} (25 cm and 4.6 mm i.d.) with 5 µm particle size. The mobile phase (43:47) was acetonitrile and sodium perchlorate buffer (pH 3.1 ± 0.3) at a flow rate of 1.7 mL/min and run time was 14 min. A 20 µL volume was injected into the system and the eluent was monitored at 210 nm. The retention time of ramipril was 5.9 ± 0.05 min at ambient temperature. The mean calibration curve was given by the equation $y = 22{,}646x$, with a correlation coefficient, $r^2 = 0.9998$, where y represents area under the curve and x the concentration in µg/mL.

2.10. Bioavailability study of ramipril in rats

Approval to carry out *in vivo* study was obtained from Jamia Hamdard, Institutional Animal Ethics Committee and their guidelines were followed for the studies. The nanoemulsion formulation (TF3), which showed the highest release profile of drug based on *in vitro* studies, was taken for *in vivo* studies. The animals used for *in vivo* experiments were adult Wistar male albino rats (250–300 g). Three groups were made for the study, and six rats were kept in each group. The animals were kept under standard laboratory conditions, temperature at 25 ± 2 °C and relative humidity (55 ± 5%). The animals were housed in polypropylene cages, three per cage, with free access to standard laboratory diet (Lipton feed, Mumbai, India) and water *ad libitum*. The formulations (nanoemulsion, marketed capsule and drug suspension) were given orally using oral feeding sonde. Dose for the rats was calculated based on the weight of the rats (0.45 mg of ramipril per kg body weight) according to the surface area ratio [37]. The rats were anesthetized using ether and blood samples (0.5 mL) were withdrawn from the tail vein of rat at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60 and 72 h in vacutainer tubes, mixed and centrifuged at 5000 rpm for 20 min. The plasma was separated and stored at −21 °C until drug analysis was carried out using LC/MS/MS.

2.11. LC/MS/MS analysis of ramiprilate (active metabolite of ramipril) in plasma

Analysis was carried out at Ranbaxy Research Laboratory, Haryana, India, using in-house developed method.

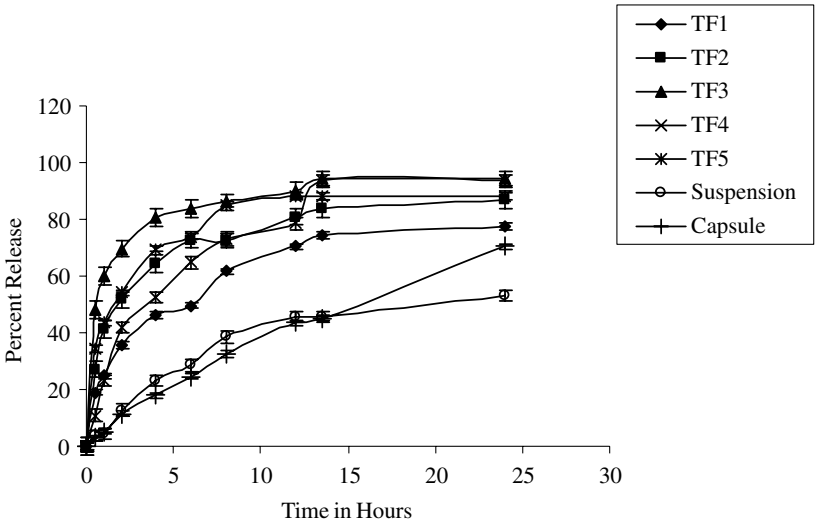

Fig. 6. Dissolution profile of ramipril (mean percent release ± SD, $n = 3$) from five different nanoemulsion formulations (TF1 to TF5), conventional capsule and drug suspension.

All samples were vortexed to ensure complete mixing of contents. An aliquot amount of each sample (200 μL) was pipetted out separately into polypropylene tubes, 30 μL of trandolaprilat stock dilution mixture (0.3 μg/mL) as internal standard (IS) and 150 μL of 5% formic acid solution were added. Solid-phase extraction cartridge (OASIS HLB 30 ng/1 cc) was conditioned using 1 mL methanol and 1 mL HPLC water on a positive pressure solid phase extraction unit. Samples were loaded onto the cartridge followed by washing with 1 mL of HPLC grade water twice. Cartridge was dried under positive pressure. Samples were eluted with 300 μL of mobile phase and transferred to the vials for analysis on HPLC MS/MS unit. The integrated system used was Shimadzu Controller (Shimadzu, Japan), having software, Analyst 1.4. The column used was Chromolith Speed Rod RP-18e (50 × 4.6 mm) 5μ and the mobile phase was 0.1% formic acid solution: Methanol (20:80 v/v). Flow rate of mobile phase was 0.7 mL/min, with a column oven temperature of 35 ± 1.0 °C and sample cooler temperature, 10 ± 1.0 °C. Injection volume was 25 μL. The retention time for ramiprilat and trandolaprilat was 1.09 ± 0.05 min and 1.15 ± 0.05 min, respectively. Detection in MS–MS was on ramiprilat 389.3 (parent) and 206.0 (product) m/z and trandolaprilat, 403.2 (parent) and 170.0 (product) m/z. The mean calibration curve was given by the equation $y = 0.02x$, correlation coefficient was $r^2 = 0.9986$, where y represents peak area ratio and x represents concentration of the respective ramiprilat.

2.12. Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated by non-compartmental analysis also called as Model independent analysis using WinNonLin version 4.0 (Pharsight Corp., Mountain View, CA). All pharmacokinetic (PK) parameters (t_{max}, C_{max}, $AUC_{0\to t}$, $AUMC_{0\to t}$ and $MRT_{0\to t}$) were calculated individually for each subject in the group and the values were expressed as means ± SD. The software estimates area under concentration time curve ($AUC_{0\to t}$) according to linear/log trapezoidal method. The PK data between different formulations were compared for statistical significance by the one-way ANOVA followed by Tukey–Kramer multiple comparisons test using GraphPad Instat software (GraphPad Software Inc., CA, USA).

3. Results and discussion

3.1. Component selection

The important criterion for selection of the materials was that all the components are pharmaceutically acceptable for oral administration and fall under GRAS (Generally regarded as safe) category. The higher solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in solubilized form. If the surfactant or cosurfactant is contributing to drug solubilization, there could be a risk of precipitation, as dilution of nanoemulsion in GIT will lead to lowering of solvent capacity of surfactant or cosurfactant [21,38]. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC under the prevailing conditions of temperature, pH and ionic strength [23]. Thus, for the present study, one oil from different categories such as long chain triglyceride, medium chain triglyceride as well as synthetic monoglyceride oils was selected, so that highest solubility of ramipril could be achieved.

Safety is a major determining factor in choosing a surfactant as large amounts of surfactants may cause GI irritation. Nonionic surfactants are less toxic than ionic surfactants. Nonionic 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 [24]. An important criterion for selection of the surfactants is that the required HLB value to form o/w nanoemulsion is greater than 10 [3]. The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water [3]. In the present study, two surfactants namely Labrasol and Tween 80 were selected having HLB values 14 and 15, respectively. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of single surfactant, usually necessitating the addition of a cosurfactant. The presence of cosurfactants 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 [20,23]. Thus, the cosurfactants selected for the study were Carbitol and Plurol oleique that again are nonionic surfactants.

Ramipril is a very sensitive and unstable molecule. Formulation of ramipril dosage form leads to very rapid decrease in the assay of ramipril due to increase in diastereomers as related substances [39]. It is well known that ramipril degrades when compressed or mixed with particular excipients, at higher temperature, in presence of moisture and in alkaline pH [40,41]. Therefore, it was necessary to check the stability of ramipril in nanoemulsion formulation. Since the drug is highly lipophilic, it was presumed that keeping it in lipophilic environment might increase its stability. It was observed from the stability studies done in our laboratory using standard buffer solution, pH 1.2, to pH 6 and distilled water as an aqueous phase in nanoemulsion formulation; there was less degradation of ramipril using standard buffer solution pH 5, as compared to other standard buffer solutions or distilled water (unpublished data). Therefore, in the present study, standard buffer solution of pH 5 was used as an aqueous phase for phase diagram construction as well as in formulation development.

3.2. Screening of components

The solubility of ramipril in different oils and water was determined (Table 2). The solubility of ramipril was found

Table 2
Solubility of ramipril in various oils and water

Oils	Solubility (mg/mL), mean ± SD ($n = 3$)
Safsol 218	199.33 ± 4.04
Triacetin	41.90 ± 1.68
IPM	15.83 ± 0.76
Labrafac	9.13 ± 0.51
Castor oil	9.33 ± 1.33
Water	0.09 ± 0.01

to be highest in Sefsol 218 (199.33 ± 4.04 mg/mL) as compared to other oils while in water it was 0.09 ± 0.01 mg/mL. This may be attributed to the polarity of the poorly water soluble drugs that favour their solubilization in small/medium molecular volume oils such as medium chain triglycerides or mono- or diglycerides [21]. Thus, Safsol 218 was selected as the oil phase for the development of the formulation.

3.3. Pseudo-ternary phase diagram study

Constructing phase diagrams is time consuming, particularly when the aim is to accurately delineate a phase boundary [21]. Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous [42]. The relationship between the phase behaviour of a mixture and its composition can be captured with the aid of a phase diagram [21]. Sefsol 218 (oil), Labrasol, Tween 80 (surfactants) and Carbitol, Plurol oleique (cosurfactant) were put in Groups I–IV (Table 1) to study the phase diagrams in detail. Pseudo-ternary phase diagrams were constructed separately for each group (Figs. 1–4), so that o/w nanoemulsion regions could be identified.

In Fig. 1(a) (Smix ratio 1:0) it can be observed that when labrasol was used alone without cosurfactant, very low amount of oil (12% w/w) could be solubilized at a high concentration (55% w/w) of surfactant. As the concentration of surfactant increased solubilization of oil decreased. When cosurfactant was added with surfactant in equal amount [Smix ratio 1:1{Fig. 1(b)}], the nanoemulsion region in the phase diagram increased and the oil solubilized up to 25% w/w with the Smix concentration of 50% w/w. When cosurfactant concentration was further increased to Smix ratio 0.5:1 {Fig. 1(c)}, it was observed that the nanoemulsion area decreased as compared to Smix ratio 1:1. Further cosurfactant concentration was increased to make Smix ratio 1:3 in which very small area of nanoemulsion was obtained which was unstable and showed phase separation after 24 h (data not shown). When surfactant concentration was increased with respect to cosurfactant [Smix ratio 2:1 {Fig. 1(d)}], it was seen that nanoemulsion area decreased as compared to 1:1 ratio and here also only up to 25% w/w oil could be solubilized with a surfactant concentration of 50% w/w. When the surfactant concentration was further increased to 3 parts is to 1 part of cosurfactant {Fig. 1(e)}, the nanoemulsion area decreased further and maximum amount of oil that could be solubilized was 16% w/w and that too at a higher concentration of Smix (55% w/w). It can be observed that the formulations prepared from phase diagrams in which the nanoemulsion area was extended towards aqueous rich apex could be diluted to a larger extent.

In case of Group II {Figs. 2(a–e)}, small nanoemulsion area was obtained as compared to Group I. In Smix ratio 1:1{Fig. 2(a)}, there was small increase in nanoemulsion area as compared to 1:0 {Fig. 1(a)}. Although nanoemulsion gel area was observed at lower concentration of surfactant using 5% w/w of oil whereas at higher oil concentration emulsion or phase separation was obtained. The maximum amount of oil that could be solubilized in this ratio was 15% w/w at 45% w/w of Smix. As cosurfactant concentration was increased from 1:1 to 1:3 {Figs. 2(b and c)}, the nanoemulsion region reduced. Similarly, when surfactant concentration was increased from 1:1 to 2:1 {Fig. 2(d)}, less solubilization of oil (13% w/w) in nanoemulsion region was achieved using 53% w/w of Smix. When further surfactant concentration was increased to 3:1 ratio (Fig. 2(e)), nanoemulsion region further reduced, making just 10% w/w oil soluble at 50% w/w Smix. As can be seen in the phase diagram, extensive dilution of the formulation is possible in 1:1, 2:1 and 3:1 ratios of Smix.

The combination of Sefsol 218, Tween 80 and Carbitol as oil, surfactant and cosurfactant (Group III) was promising due to extensive area of nanoemulsion found as shown in Figs. 3(a–g). When surfactant was used alone (Smix ratio 1:0, Fig. 3(a)), large nanoemulsion gel area (not shown in figure) was obtained while small o/w nanoemulsion region was found towards aqueous rich apex and Smix rich apex. The maximum concentration of oil that could be solubilized was 28% w/w by using 41% w/w of Smix. When cosurfactant was added along with surfactant in equal ratio {1:1, Fig. 3(b)}, the whole area which was nanoemulsion gel in 1:0 changed to easily flowable o/w nanoemulsion area. This may be attributed to the fact that the addition of cosurfactant may lead to greater penetration of the oil phase in the hydrophobic region of the surfactant monomers thereby further decreasing the interfacial tension, which will lead to increase in the fluidity of the interface thus increasing the entropy of the system. [21,43]. The maximum oil that could be solubilized was 28% w/w using 33% w/w of Smix. When cosurfactant concentration was doubled, Smix ratio 1:2 {Fig. 3(c)}, the maximum amount of oil that could be solubilized was 30% w/w with 44% w/w of Smix whereas the total area of nanoemulsion decreased as compared to 1:1. When further cosurfactant concentration was increased to 1:3 {Fig. 3(d)}, nanoemulsion area decreased considerably making just 25% w/w oil solubilized with 50% w/w Smix. In contrast, when surfactant concentration was increased as compared to cosurfactant, Smix ratio 2:1{Fig. 3(e)}, the concentration of oil that could be solubilized was increased up to 32% w/w using

Smix concentration 32% w/w only but the nanoemulsion region decreased as compared to 1:1. A small nanoemulsion gel area was also observed which may be due to increased character of surfactant. When further surfactant concentration was increased to 3:1{Fig. 3(f)}, and 4:1{Fig. 3(g)}, nanoemulsion area in the phase diagrams slowly decreased with increase in nanoemulsion gel area. The maximum concentration of oil that could be solubilized in Smix ratio 4:1 was 18% w/w with a very high Smix concentration of 62% w/w.

In case of Group IV, {Figs. 4(a–e)} the o/w nanoemulsion area was limited. This combination is good for making nanoemulsion gel as a large area of this could be achieved in these phase diagrams (data not shown). Thus, this group was dropped from further study.

While studying the phase diagrams (Figs 1–4), it can be seen that the free energy of nanoemulsion formation can be considered to depend on the extent to which the surfactant lowers the surface tension of the oil–water interface and the change in dispersion entropy [21]. Thus, a negative free energy of formation is achieved when large reduction in surface tension is accompanied by significant favourable entropic changes. In such case nanoemulsion formation is spontaneous and the resulting dispersion is thermodynamically stable [21,42]. Therefore, the system that has potential for oral drug delivery is the one in which surfactant or Smix concentration used should be able to increase the dispersion entropy, reduce the interfacial tension, increase the interfacial area, lower the free energy of the system to a very low value with the minimum concentration (weight ratio), resulting in spontaneous dispersion which is thermodynamically stable.

3.4. Selection of formulations from phase diagrams

It is well known that large amounts of surfactants cause GI irritation [21,44]; therefore, it is important to determine the surfactant concentration properly and use minimum concentration in the formulation. As per our knowledge, nobody has reported until date the basis of selecting different nanoemulsion or microemulsion formulations from the phase diagram, as hundreds of formulations can be prepared from nanoemulsion region of the diagram. While going through different pseudo-ternary phase diagrams (Figs. 1–4), oil could be solubilized up to the extent of 35% w/w. Therefore, from each phase diagram different concentrations of oil, which formed nanoemulsions, were selected at a difference of 5% (10, 15, 20, 25 and 30%) so that maximum formulations could be selected covering the nanoemulsion area of the phase diagram (Tables 3–5). For each percentage of oil selected, only those

Table 3
Thermodynamic stability and dispersibility test of different formulations selected from Group I (Figs. 1a–e) at a difference of 5% w/w of oil

Smix ratio (S:CoS)	Percentage w/w of different components in formulation			Observations based on the preparation, thermodynamic stability studies and dispersibility tests				Inference
	Oil	Smix	Aqueous	H/C	Cent.	Freez. Tha.	Disperse.	
1:0 (Fig. 1a)	10	50	40	√	√	√	Grade D	Failed
1:1 (Fig. 1b)	10	40	50	√	√	√	Grade C	Failed
	15	46	39	√	√	√	Grade E	Failed
	20	51	29	√	√	√	Grade E	Failed
	25	50	25	√	√	√	Grade E	Failed
0.5:1 (Fig. 1c)	10	50	40	√	√	√	Grade E	Failed
	15	50	35	√	√	√	Grade E	Failed
	20	45	35	√	X	–	–	Failed
	25	50	25	√	√	√	Grade E	Failed
2:1 (Fig. 1d)	10	40	50	√	√	√	Grade C	Failed
3:1 (Fig. 1e)	10	47	43	√	√	√	Grade E	Failed

Heating cooling cycle (H/C), centrifugation (Cent.), freeze-thaw cycle (Freez. Tha.), dispersibility test (Disperse.).

Table 4
Thermodynamic stability and dispersibility test of different formulation selected from Group II (Figs. 2 a, d, and e) at a difference of 5% w/w of oil

Smix ratio (S:CoS)	Percentage w/w of different components in formulation			Observations based on the preparation, thermodynamic stability studies and dispersibility tests				Inference
	Oil	Smix	Aqueous	H/C	Cent.	Freez. Tha.	Disperse.	
1:1 (Fig. 2a)	10	55	35	√	X	–	–	Failed
	15	55	30	√	X	–	–	Failed
2:1 (Fig. 2d)	10	50	40	√	√	X	–	Failed
3:1 (Fig. 2e)	10	47	45	√	X	–	–	Failed

Heating cooling cycle (H/C), centrifugation (Cent.), freeze thaw cycle (Freez. Tha.), dispersibility test (Disperse.).

Table 5
Thermodynamic stability and dispersibility test of different formulation selected from Group III (Figs. 3a–g) at a difference of 5% w/w

Smix ratio (S:CoS)	Percentage w/w of different components in formulation			Observations based on the preparation, thermodynamic stability studies and Dispersibility tests				Inference
	Oil	Smix	Aqueous	H/C	Cent.	Freez. Tha.	Disperse.	
1:0 (Fig. 3a)	10	67	23	√	√	√	Grade A	Passed
	15	60	25	√	√	√	Grade A	Passed
	20	49	31	√	√	√	Grade D	Failed
1:1 (Fig. 3b)	10	50	40	√	√	√	Grade A	Passed
	15	35	50	√	√	√	Grade D	Failed
	20	36	44	√	√	√	Grade B[a]	Passed
	25	35	40	√	√	√	Grade D	Failed
0.5:1(Fig. 3c)	10	35	55	√	√	√	Grade B	Passed
	15	40	45	√	√	√	Grade A[a]	Passed
	20	45	35	√	√	√	Grade B	Passed
	25	43	32	√	√	√	Grade E	Failed
	30	44	26	√	√	√	Grade D	Failed
1:3 (Fig. 3d)	10	40	50	√	√	√	Grade A	Passed
	15	45	40	√	√	√	Grade B	Passed
	20	50	30	√	√	√	Grade D	Failed
	25	50	25	√	√	√	Grade D	Failed
2:1 (Fig. 3e)	10	35	55	√	√	√	Grade A[a]	Passed
	15	39	46	√	√	√	Grade B	Passed
	20	38	42	√	√	√	Grade B	Passed
	25	37	38	√	√	√	Grade B[a]	Passed
	30	35	35	√	√	√	Grade B[a]	Passed
3:1 (Fig. 3f)	10	36	54	√	√	√	Grade A	Passed
	15	50	35	√	√	√	Grade A	Passed
	20	50	30	√	√	√	Grade A	Passed
	25	33	42	√	√	√	Grade D	Failed
	30	35	35	√	√	√	Grade E	Failed
4:1 (Fig. 3g)	10	45	45	√	√	√	Grade A	Passed
	15	59	26	√	√	√	Grade A	Passed

Heating cooling cycle (H/C), centrifugation (Cent.), freeze thaw cycle (Freez. Tha.), dispersibility test (Disperse.).
[a] Optimized formulas having the least Smix concentration.

formulations were taken from the phase diagram, which used minimum concentration of Smix.

There was no effect seen in the phase behaviour and nanoemulsion area of phase diagrams when ramipril (5 mg) was incorporated in the formulations which was expected as the formation and stability of nano- and microemulsions consisting of nonionic components is not affected by the pH and or ionic strength [20–22,45].

3.4.1. Thermodynamic stability studies

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nano- or microemulsion from emulsions that have kinetic stability and will eventually phase separate [21,46]. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for dispersibility test. It was observed that formulation prepared from Sefsol 218, Labrasol and Plurol oleique did not pass the thermodynamic stress tests and thus were dropped for further study (Table 4).

3.4.2. Dispersibility test

When infinite dilution is done to nanoemulsion formulation, there is every possibility of it to phase separate, leading to precipitation of a poorly soluble drug as nanoemulsions are formed at a particular concentration of oil, surfactant and water. For oral nanoemulsions the process of dilution by the GI fluids will result in the gradual desorption of surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC [21].

In the present study, we used distilled water as a dispersion medium because it is well reported that there is no significant difference in the nanoemulsions prepared using nonionic surfactants, dispersed in either water or simulated gastric or intestinal fluid [21,34,44]. Formulations in Group III (Table 5) that passed dispersibility test in Grade A and

B were taken for further study, as Grade A and B formulations will remain as nanoemulsions when dispersed in GIT. All the formulation in the Group I and III (Tables 3 and 5), that were falling in Grade C, D and E of dispersibility tests were discarded for further study. Formulation falling in Grade C could be recommended for self-emulsifying drug delivery formulation. Keeping the criteria of increasing oil concentration and minimum amount of surfactant used for its solubilization, one formulation for each percent of oil (10%, 15%, 20%, 25% and 30%) was selected from Group III (Table 5) irrespective of the Smix ratio used for that percent of oil. Optimized formulations (Table 6) were taken for *in vitro* release study, globule size and viscosity determination.

3.5. Globule size analysis

The globule size analysis of the optimized formulations was done using Zetasizer. The globule size increased with increase in concentration of oil in the formulations (Table 7). The mean globule size of the formulation, TF1, containing 10% of oil was 71.6 nm while as formulation, TF5, containing 30% of oil was 92.1 nm. The difference in the droplet size between the formulation is not statistically significant ($p > 0.05$). There is only a marginal difference in the mean globule size of formulations but polydispersity was minimum in the case of formulation, TF3, containing 20% of oil suggesting uniformity in the globule size (80.9 nm) of the formulation.

3.6. Viscosity determination

The viscosity of the optimized formulations was determined. The values are shown in Table 7. It was observed that the viscosity of all the formulations is less than 21 cP. Formulation, TF3, has the minimum viscosity (10.68 ± 0.99 cP) which is highly significant ($p < 0.01$) as compared to the other formulations.

3.7. Transmission electron microscopy

The nanoemulsion appears dark and the surroundings are bright (Fig. 5), a ''positive'' image is seen using TEM. Some droplet sizes are measured using TEM, as it is capable of point-to-point resolution. The droplet size is in agreement with the results obtained from droplet size analysis using zetasizer.

Table 7
Mean globule size, polydispersity values and mean viscosity ± SD ($n = 3$) of the nanoemulsion formulations

Formulation code	Mean globule size (nm)	Polydispersity	Viscosity (cP)
TF1	71.6	0.651	20.55 ± 1.01
TF2	75.3	0.387	15.00 ± 0.91
TF3	80.9	0.271	$10.68^{a} \pm 0.99$
TF4	87.6	0.404	14.25 ± 1.18
TF5	92.1	0.429	16.08 ± 1.21

[a] $p < 0.01$ when compared to other formulation.

3.8. In vitro dissolution study

Dissolution studies were performed to compare the release of drug from five different nanoemulsion formulations (TF1 to TF5), conventional capsule formulation and simple drug suspension, having same quantity (5 mg) of ramipril. The release of drug from nanoemulsion formulations was highly significant ($p < 0.01$) when compared to marketed capsule and drug suspension (Fig. 6). The highest release i.e., $95 \pm 2.5\%$ was obtained in case of formulation TF3. Out of 95%, 60% drug release was obtained in first hour of study itself compared to capsule and suspension, which released less than 5% of the drug. This is because of small globule size, and eventually higher surface area in case of nanoemulsions, which permit faster rate of drug release. Interesting result was that the release from TF1 and TF2 was significantly ($p < 0.01$) less than TF3, in spite of TF3 having higher globule size although not statistically significant ($p > 0.05$). This may be attributed to the fact that the concentration of oil in TF3 is 20% w/w; therefore, the total number of globules formed will be more than that in formulation TF1 and TF2. In addition, the variation in globule sizes in TF3 was less than TF1 and TF2 because the polydispersity is minimum (Table 7). Thus, the drug has larger surface area for release and low viscosity in TF3 than TF1 and TF2. In spite of increased oil concentration in formulation TF4 and TF5 (25% w/w and 30% w/w), the drug release from the formulations was significantly ($p < 0.05$) lower as compared to TF3 which may be due to higher globule size, higher polydispersity value and

Table 6
Optimized formulation selected from Group III at a difference of 5% w/w of oil having least Smix concentration for dilutable nanoemulsion formation

Oil used: Sefsol 218, Surfactant used: Tween 80, cosurfactant used: Carbitol

Smix ratio	Percentage w/w of components in nanoemulsion formulation				Oil:Smix ratio	Dispersibility test grade	Code
	Oil	S	CoS	Aqueous			
2:1	10	23.3	11.7	55	1:3.5	Grade A	TF1
0.5:1	15	13	27	44	1:2.7	Grade A	TF2
1:1	20	18	18	44	1:1.8	Grade B	TF3
2:1	25	24.7	12.3	38	1:1.48	Grade B	TF4
2:1	30	24	12	34	1:1.2	Grade B	TF5

higher viscosity (Table 7). Thus surface area for the drug release may be more in TF3 than TF4 and TF5. In addition to this the higher oil concentration may restrain the release of the drug into the medium due to high lipophilic character of ramipril (log P is 3.32) as the partitioning of drug will be more towards the oil.

Therefore, the optimized formulation, TF3, having higher drug release (95%), optimum globule size (80.9 nm), minimum polydispersity value (0.271), lower viscosity (10.68 cP), stability of nanoemulsion and drug and above all, lower surfactant concentration (36%) was selected for the *in vivo* study.

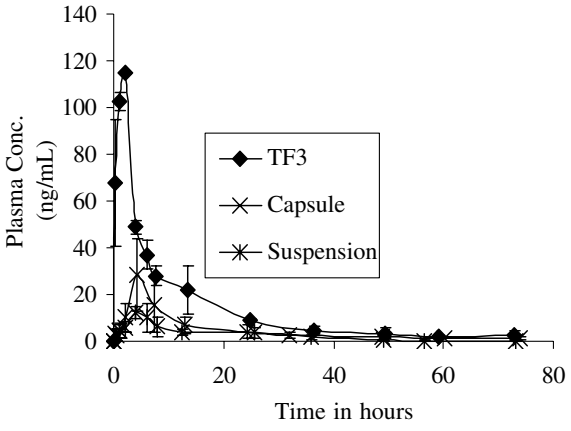

Fig. 7. Plasma concentration profile of ramiprilat after oral administration of nanoemulsion formulation (TF3), conventional capsule formulation, and dug suspension in adult Wistar male albino rats ($n = 6$ and dose 0.45 mg/kg).

3.9. Bioavailability study

The *in vivo* study was performed to quantify ramiprilat, an active metabolite, after oral administration of ramipril formulations. The plasma profiles of ramiprilat in adult male albino Wistar rats following oral administration of the nanoemulsion (TF3), marketed capsule and drug suspension of ramipril were compared. The plasma concentration profile of ramiprilat for nanoemulsion represents significantly greater improvement of drug absorption than the marketed formulation or simple drug suspension (Fig. 7).

As can be seen in Tables 8 and 9, t_{max} and C_{max} of TF3 were 2.17 ± 0.40 h and 114.3 ± 7.04 ng/mL, respectively, as compared to those of capsule which were 5.33 ± 1.7 h and 32.76 ± 11.11 ng/mL and drug suspension 4.67 ± 1.20 h and 14.39 ± 1.90 ng/mL, respectively. Statistically, the difference in t_{max} of TF3 was highly significant ($p < 0.01$) when compared to t_{max} of capsule (Table 8) and drug suspension (Table 9). The difference in C_{max} of TF3 formulation was extremely significant ($p < 0.001$) when compared with the tablet formulation and drug suspension. It was also observed that $AUC_{0\to t}$ and $AUMC_{0\to t}$ of TF3 formulation were 1000.8 ± 172.4 ng h/mL and 14050 ± 4239 ng h/mL, respectively, and thus the

Table 8
Relative bioavailability and mean ± SD pharmacokinetic parameters of ramiprilat when ramipril nanoemulsion and capsule formulations were orally administered to male albino rats ($n = 6$)

Formulation	t_{max}[a] (h)	C_{max}[b] (ng/ml)	$AUC_{0\to t}$[c] (ng h/ml)	$AUMC_{0\to t}$[d] (ng h/ml)	$MRT_{0\to t}$[e] (h)	Rel. BA[f] (%)
TF3	2.17** ± 0.40	114.3*** ± 7.04	1000.8*** ± 172.4	14050** ± 4239	16.82 ± 1.95	229.62
Capsule	5.33 ± 1.70	32.76 ± 11.11	339.97 ± 120.6	6457 ± 2492	18.97 ± 2.60	–

**$p < 0.01$ and ***$p < 0.001$ when compared with tablet formulation using one way ANOVA followed by Tukey–Kramer multiple comparison test.
[a] Time of peak concentration.
[b] Peak of maximum concentration.
[c] Area under the concentration time profile curve until last observation.
[d] Area under moment curve computed to the last observation.
[e] Mean residence time.
[f] Relative bioavailability.

Table 9
Relative bioavailability and mean ± SD pharmacokinetic parameters of ramiprilat when ramipril nanoemulsion and drug suspension were orally administered to male albino rats ($n = 6$)

Formulation	t_{max}[a] (h)	C_{max}[b] (ng/ml)	$AUC_{0\to t}$[c] (ng h/ml)	$AUMC_{0\to t}$[d] (ng h/ml)	$MRT_{0\to t}$[e] (h)	Rel. BA[f] (%)
TF3	2.17** ± 0.40	114.3*** ± 7.04	1000.8*** ± 172.4	14050*** ± 4239	16.82 ± 1.95	539.49
Drug suspension	4.67 ± 1.20	14.39 ± 1.90	185.51 ± 65.70	3151 ± 1582	16.70 ± 4.40	–

**$p < 0.01$ and ***$p < 0.001$ when compared with tablet formulation using one way ANOVA followed by Tukey–Kramer multiple comparison test.
[a] Time of peak concentration.
[b] Peak of maximum concentration.
[c] Area under the concentration time profile curve until last observation.
[d] Area under moment curve computed to the last observation.
[e] Mean residence time.
[f] Relative bioavailability.

Table 10
Mean ± SD, pharmacokinetic parameters of ramiprilat when ramipril dosage forms were orally administered to male albino rats ($n = 6$)

PK	Initial rapid profile			Apparent elimination profile			Last elimination profile		
	TF3	Caps	Susp[a]	TF3	Caps	Susp	TF3	Caps	Susp
K_{el} or λz[b] (h^{-1})	0.1494 ± 0.03	0.0508 ± 0.02	0.0496 ± 0.02	0.06 ± 0.01	0.0419 ± 0.04	0.0786 ± 0.06	0.0085 ± 0.004	0.0237 ± 0.010	0.1715 ± 0.200
$t_{1/2}$[c] (h)	4.74 ± 0.8	15.10 ± 5.90	17.10 ± 10.5	11.24 ± 2.20	26.24 ± 18.3	11.67 ± 6.80	101.8 ± 53.9	32.19 ± 13.6	8.90 ± 9.30
$AUC_{0\to z}$[d] (ng h/ml)	1008.5 ± 170.8	372.17 ± 133.7	187.91 ± 66.9	1039 ± 189	386 ± 96	187.1 ± 65	1353.3 ± 374.6	431.45 ± 138.0	198.33 ± 89.60
$AUMC_{0\to z}$[e] (ng h/ml)	15537 ± 5301	9636 ± 4393	3375.28 ± 1762	17475 ± 5743	11909 ± 933	3287 ± 1630	93925 ± 58869	12996 ± 2380	3889 ± 1738
$MRT_{0\to z}$[f] (h)	14.94 ± 2.59	25.50 ± 5.91	17.60 ± 4.60	16.51 ± 2.60	32.37 ± 9.20	17.34 ± 4.80	67.10 ± 31.90	30.82 ± 4.40	19.63 ± 0.10

[a] Drug suspension.
[b] First-order rate constant associated with the terminal (log-linear) portion of the curve.
[c] Half life.
[d] Area under curve extrapolated to infinity.
[e] Area under movement curve when the time concentration curve is extrapolated to infinity.
[f] Mean residence time when the drug concentration profile is extrapolated to infinity.

difference was extremely significant ($p < 0.001$) as compared to $AUC_{0\to t}$ (339.97 ± 120.6 ng h/mL) and highly significant ($p < 0.01$) $AUMC_{0\to t}$ (6457 ± 2492 ng h/mL) of capsule formulation (Table 8). Both the values of TF3 were extremely significant ($p < 0.001$) as compared to drug suspension (Table 9). The difference in the values of $MRT_{0\to t}$ is not significantly different ($p > 0.05$) when the nanoemulsion, capsule or suspension was compared, as there is no change in the intrinsic properties of the drug when it is formulated into different formulations. The relative bioavailability of TF3 to that of conventional capsule was 229.62% whereas to that of drug suspension it was 539.49%.

The interesting observation seen in the concentration time profile of TF3 and capsule formulations was the triphasic decline in the ramiprilat concentration (Fig. 7), which has been attributed in the literature [26,27,47] as the initial rapid decline, middle apparent elimination and terminal elimination phase. The initial rapid decline, which represents distribution of the drug into a large peripheral compartment with subsequent binding to both plasma and tissue ACE, had a half life of 4.74 h, in TF3 (Table 10). Due to its potent binding to ACE and slow dissociation from enzyme, ramiprilat shows two elimination phases. The apparent elimination phase could be the clearance of free ramiprilat and had a half life of 11.24 h with TF3 formulation. The terminal elimination phase has a prolonged half life (101.8 h with nanoemulsion) and possibly represents binding/dissociation kinetics of the ramiprilat/ACE complex. Levitt and Schoemaker [27] in their research paper report that there are two binding sites per ACE (high affinity ”C”, lower affinity “N”) that have sub-nanomolar affinities and dissociation rates of hours. The ACE enzyme in plasma and tissue has a very high affinity for the ACE inhibitors. This produces extremely nonlinear kinetics as the concentration falls from high concentrations when most of the drug is free, to low concentrations when most of the drug is bound to ACE [25,27]. Although it is known that more than 90% of the total ACE is in the tissues, the quantitative distribution of tissue ACE is not well characterized [27].

The PK profiles for all the three phases are given in Table 10, which clearly shows the enhanced bioavailability of ramipril nanoemulsion over that of marketed capsule and drug suspension. The drug present in the solubilized form as nanolipid globules provides large interfacial area for drug absorption [10,15]. Furthermore, the presence of a surfactant, Tween 80, in nanoemulsion system in the GI tract might have caused changes in membrane permeability by the inhibition of an apocaly polarized efflux system, which could lead to enhancement of the oral absorption of drug [48].

4. Conclusion

Based on higher drug release, optimum globule size, minimum polydispersity, lower viscosity, lower

surfactant concentration, higher solubility as well as higher bioavailability without variable absorption has been optimized as nanoemulsion formulation of ramipril containing Sefsol 218 (20% w/w), Tween 80 (18% w/w) and Carbitol (18% w/w) as oil, surfactant and cosurfactant, respectively. The *in vivo* studies revealed significantly greater extent of absorption than the conventional capsule formulation. The absorption of ramiprilat from ramipril nanoemulsion resulted in 2.94-fold increase in bioavailability as compared to conventional capsule and 5.4-fold to that of drug suspension. Our studies illustrated the potential use of ramipril formulated as nanoemulsions, can be used as a liquid formulation for pediatric and geriatric patients as well can be formulated as SNEDDS using soft gelatin capsules as unit dosage form. Studies also showed how nanoemulsion formulation can be optimized for the delivery of hydrophobic compounds with higher drug loading, minimum surfactant concentration and proper infinite dilution can be achieved without drug precipitation.

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