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Effect of labrasol on self-nanoemulsification efficiency of ramipril nanoemulsion

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Received June 8, 2009, accepted June 19, 2009

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Pharmazie 64: 812–817 (2009) *doi: 10.1691/ph.2009.9186*

The purpose of the present investigation was to evaluate the capacity of Labrasol as surfactant for self-nanoemulsification efficiency of ramipril nanoemulsion formulation. Based on the solubility profile of ramipril, Sefsol-218, Labrasol and Carbitol were selected as oil phase, surfactant and cosurfactant, respectively. Based on the stability profile of ramipril, standard buffer solution of pH 5.0 was selected as an aqueous phase for the development of ramipril nanoemulsion formulation. Nanoemulsion formulations of ramipril were developed using an aqueous phase titration method. Pseudoternary phase diagrams were constructed to identify the nanoemulsion region. Selected formulations were subjected to different thermodynamic stability tests using centrifugation, heating cooling cycles and freeze thaw cycles. The formulations which were stable at thermodynamic stability tests were taken for self-nanoemulsification efficiency test. No creaming, cracking, coalescence or phase inversion was observed on most of the formulations upon thermodynamic stability tests. All the formulations passed self-nanoemulsification tests in grade C, D and E but not in grade A and B. Because none of the formulation passed self-nanoemulsification efficiency test in grade A and B, it was concluded that Labrasol is not suitable as surfactant for oral or self nanoemulsifying drug delivery system of ramipril.

1. Introduction

Ramipril, a potent antihypertensive pro-drug is almost completely converted to its active metabolite ramiprilat by hydrolytic cleavage of the ester group in the liver (Shafiq et al. 2007a). It is a highly lipophilic (logP 3.32), poorly soluble drug with an absolute bioavailability of 28–35 % (Griensven et al. 1995; Shafiq et al. 2007a). In recent years, much attention has been paid on lipid based formulations to improve the solubility, permeability and bioavailability of poorly soluble drug compounds. The most popular approach for solubility and bioavailability enhancement is the incorporation of the active lipophilic compound into inert lipid vehicles such as oils, lipids, surfactant dispersions, microemulsions, nanoemulsions, self-emulsifying formulations, self-microemulsifying formulations, emulsions and liposomes (Stella et al. 1978; Palin et al. 1986; Serajuddin et al. 1988; Toguchi et al. 1990; Charman et al. 1992; Constantinides 1995; Shafiq et al. 2007a, b; Shakeel et al. 2008a, b). One of the most promising technologies is the nanoemulsion or self-nanoemulsifying drug delivery system (SNEDDS), which is being applied to enhance the oral bioavailability of poorly soluble drug compounds. Nanoemulsions are thermodynamically stable, transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant molecules usually in combination with cosurfactant having the droplet size 10–100 nm (Shafiq et al. 2007a, b; Shakeel et al. 2008a, b). SNEDDS are isotropic dispersions of oil, surfactant, cosurfactant and drug that form fine oil-in-water (o/w) nanoemulsion when diluted with aqueous phases under gentle agitation (Date and Nagarsenker 2007). Nanoemulsion and SNEDDS provide ultra low interfacial tensions and large o/w interfacial areas which results in enhanced solubility as well as bioavailability of poorly soluble compounds (Shafiq et al. 2007a). Nanoemulsions and SNEDDS have also been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible (Shafiq et al. 2007a, b; Shakeel et al. 2008a, b).

Surfactant Labrasol has been investigated for the development of nanoemulsion formulations of many drugs for topical/transdermal, ocular parenteral and oral drug delivery systems. Recently self-nanoemulsification efficiency of Labrasol has been investigated for the natural drug oleanolic acid (Xi et al. 2009). But in this article self-nanoemulsification efficiency of Labrasol has been investigated along with other surfactants like Tween-80 and Cremophor-EL not alone or in combination with a suitable cosurfactant. Self-nanoemulsification efficiency of Labrasol has not been investigated for the antihypertensive drug ramipril. Therefore the aim of the present investigation was to evaluate the self-nanoemulsification efficiency of Labrasol on ramipril nanoemulsion formulation. The dose of ramipril ranges between 2.5–20 mg and a frequently prescribed dose is 5 mg for the adult. Therefore, a 5 mg dose of ramipril was selected for the development of nanoemulsion formulation.

2. Investigations, results and discussion

The important criterion for selection of the materials was that all the components are pharmaceutically acceptable for oral administration and fall under the GRAS (Generally regarded as safe) category. Both long and medium chain triglyceride oils with different degrees of saturation have been used for the design of nanoemulsion formulations. 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 the highest solubility of ramipril could be achieved. Edible oils, which could represent the logical and preferred lipid excipient choice for the development of nanoemulsions, are not frequently selected 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 (Kimura et al. 1994; Constantinides 1995; Hauss et al. 1998). They offer formulative and physiological advantages and their degradation products resemble the natural end products of intestinal digestion. Novel semisynthetic medium chain derivatives, which can be defined as amphiphilic compounds with surfactant properties, are progressively and effectively replacing the regular medium chain triglyceride oils (Karim et al. 1994; Constantinides 1995). Therefore, in the present study a wide range of oils has been selected in order to get the highest solubility of ramipril. 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 form the correct curvature at the interfacial region for the desired nanoemulsion type. An important criterion for selection of the surfactants is that the required HLB value to form o/w nanoemulsion is greater than 10 (Kommuru et al. 2001). Safety is another 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. Nonionic surfactants typically have lower critical micelle concentrations (CMCs) than their ionic counterparts. O/W nanoemulsions dosage forms for oral or parenteral use based on nonionic surfactants are likely to offer *in vivo* stability (Kawakami et al. 2002b). Therefore, for the present study a wide range of nonionic surfactants has been selected having higher HLB value.

The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water (Lawrence and Rees 2000). 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 decrease 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 (Kawakami et al. 2002a).

The solubility of ramipril in different oils, surfactants, cosurfactants and distilled water is given in Table 1. The solubility of ramipril was found to be highest in Sefsol 218 (199.33 ± 4.04 mg/ml) as compared to other oils while in distilled water it was found to be 0.09 ± 0.01 mg/ml. This may be attributed to the polarity of the poorly soluble drugs that favor their solubilization in small/medium molecular volume oils such as medium chain mono or di or triglycerides (Lawrence and Rees 2000). 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 (Lawrence and Rees 2000). 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 (Kawakami et al. 2002a).

Solubility of ramipril was found to be higher in Labrasol (33.80 mg/ml), hence it was selected as surfactant for nanoemulsion development. Similarly, solubility in cosurfactants was found to be higher in Carbitol (36.57 mg/ml), hence Carbitol was selected as cosurfactant.

Based on the highest stability of ramipril, standard buffer solution of pH 5.0 was selected as an aqueous phase (Shafiq and Shakeel 2008).

Constructing phase diagrams is time consuming, particularly when the aim is to accurately delineate a phase boundary. Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form a nanoemulsion or microemulsion is very low, the formation is thermodynamically spontaneous (Craig et al. 1995). The systems were observed for visual clarity and flow. Those which did not show a change in the meniscus after tilting to an angle of 90° were classified as gels. After taking observation, pseudo ternary phase diagrams were constructed based on the observations marked during aqueous phase titration. Phase diagrams were constructed separately for each S_{mix}, so that o/w nanoemulsion regions could be identified. In the phase diagrams (Fig. a–e) only the o/w nanoemulsion region is shown. Other phases are not shown due to potential overcrowding of the diagrams as we are interested only in the o/w nanoemulsion part of the phase diagram for our formulation development.

In Fig. a (S_{mix} ratio 1:0), it was observed that when Labrasol was used alone without cosurfactant, a very low amount of oil (12% w/w) was solubilized at a higher 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 [S_{mix} ratio 1:1(Fig. b)], the nanoemulsion region in the phase diagram increased and the oil solubilized up to 25% w/w with the S_{mix} concentration of 50% w/w. When cosurfactant concentration was further increased to S_{mix} ratio 0.5:1 (Fig. c), it was observed that the nanoemulsion area decreased as compared to S_{mix} ratio 1:1. Further cosurfactant concentration was increased to make S_{mix} 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 [S_{mix} ratio 2:1 (Fig. d)], it was found that nanoemulsion area decreased as compared to 1:1 ratio and here also only up to 25% w/w oil was 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. 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 S_{mix} (55% w/w). It was 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.

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 micro emulsion from emulsions that have kinetic stability and will eventually phase separate (Lawrence and Rees 2000). Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw

Table 1: Solubility of ramipril in various oils, surfactants, cosurfactants and distilled water at 25 °C

Components	Solubility mg/ml			Mean ± SD (n = 3)
	Sample 1	Sample 2	Sample 3	
Oils				
Sefsol 218	203	195	200	199.33 ± 4.04
Triacetin	43.2	40	42.5	41.90 ± 1.68
IPM	16.5	15	16	15.83 ± 0.76
Labrafac	9.7	9	8.7	9.13 ± 0.51
Castor oil	10.8	8.2	9	9.33 ± 1.33
Surfactants				
Labrasol	33.8	32.7	34.9	33.80 ± 1.10
Labrafil	10	8	12	10.00 ± 2.00
HCO 60	10	12	10	10.67 ± 1.15
Cosurfactants and distilled water				
Plurol oleique	15	19	20	18.00 ± 2.65
Carbitol	36.3	38.1	35.3	36.57 ± 1.42
PEG	8	5.9	7.6	7.17 ± 1.12
Distilled Water	0.1	0.08	0.1	0.09 ± 0.01

Table 2: Thermodynamic stability and self-nanoemulsification efficiency test of different formulations selected at a difference of 5% w/w of oil

Oil used: Sefsol 218, Surfactant used: Labrasol, Cosurfactant used: Carbitol								
S_{mix} ratio (S:CoS)	% w/w of different components in formulation			Observations based on the preparation, thermodynamic stability and self-nanoemulsification tests				Infer
	Oil	S_{mix}	Aque	H/C	Cent	Freez	SNET	
1:0 (Fig. a)	10	50	40	√	√	√	Grade D	Failed
	10	55	35	√	√	√	Grade D	Failed
1:1 (Fig. b)	10	40	50	√	√	√	Grade C	Failed
	10	45	45	√	√	√	Grade C	Failed
	10	50	40	√	√	√	Grade D	Failed
	15	43	42	X	-	-	-	Failed
	15	46	39	√	√	√	Grade E	Failed
	15	53	32	√	√	√	Grade E	Failed
	20	48	32	X	-	-	-	Failed
	20	51	29	√	√	√	Grade E	Failed
	20	55	25	√	√	√	Grade E	Failed
	25	50	25	√	√	√	Grade E	Failed
0.5:1 (Fig. c)	10	50	40	√	√	√	Grade E	Failed
	10	55	35	√	√	√	Grade E	Failed
	10	60	30	√	√	√	Grade E	Failed
	15	45	40	X	-	-	-	Failed
	15	50	35	√	√	√	Grade E	Failed
	15	55	30	√	√	√	Grade E	Failed
	20	42	38	X	-	-	-	Failed
	20	45	35	√	X	-	-	Failed
	20	50	30	√	√	√	Grade E	Failed
	25	50	25	√	√	√	Grade E	Failed
2:1 (Fig. d)	10	40	50	√	√	√	Grade C	Failed
	10	45	45	√	√	√	Grade C	Failed
	10	50	40	√	√	√	Grade D	Failed
3:1 (Fig. e)	10	47	43	√	√	√	Grade E	Failed
	10	50	40	√	√	√	Grade D	Failed

Aqueous phase (aque), Heating cooling cycle (H/C), Centrifugation (Cent.), Freeze thaw cycle (Freez), Self-nanoemulsification efficiency test (SNET)

cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for the self-nanoemulsification efficiency tests. From these tests it was found that most of the formulations were stable at centrifugation, heating cooling cycles and freeze thaw cycles. No creaming, cracking, coalescence or phase inversion was observed on most of the formulations as shown in Table 2.

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 aqueous phase. 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

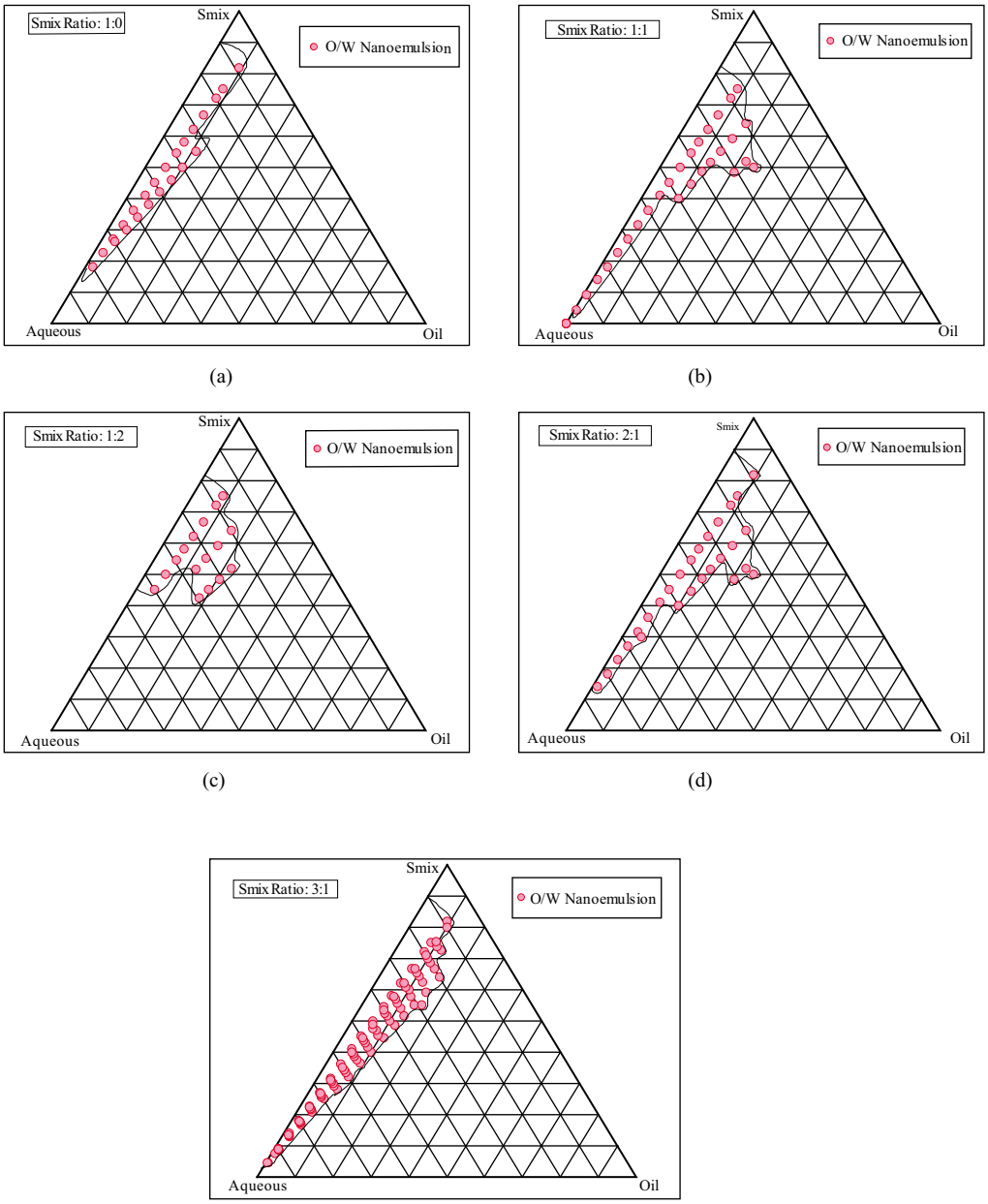

Fig.: Pseudo-ternary phase diagrams of Sefsol-218 (oil phase), Labrasol (surfactant), Carbitol (cosurfactant) and standard phosphate buffer of pH 5.0 (aqueous phase) indicating o/w nanoemulsion region at different S_{mix} ratios

by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC (Lawrence and Rees 2000). 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 non-ionic surfactants, dispersed in either water or simulated gastric or intestinal fluid (Khoo et al. 1995; Lawrence and Rees 2000; Ping et al. 2005). Formulations that passed self-nanoemulsification test in grade A and B will remain as nanoemulsions when dispersed in GI fluids. All the developed formulations were passed self-nanoemulsification test in grade C, D and E but not in grade A and B (Table 2). Therefore all formulations were not suitable for a self nanoemulsifying drug delivery system (SNEDDS) of ramipril. Because most of the formulations passed the thermodynamic stability test but none of them passed the self-nanoemulsification efficiency test in grade A and B, it was concluded that Labrasol could be suitable as surfactant for topical/transdermal, ocular or other drug delivery systems but it is not suitable for oral or SNEDDS drug delivery of ramipril. Although formulation falling in grade C could be recommended for self-emulsifying drug delivery system [SEDDS] (Shafiq et al. 2007a).

3. Experimental

3.1. Materials

Ramipril base and propylene glycol mono caprylic ester (Sefsol 218) were obtained as a kind gift samples from Ranbaxy Research Laboratory (Haryana, India) and Nikko Chemicals (Tokyo, Japan) respectively. Diethylene glycol monoethyl ether (Carbitol), isopropyl myristate (IPM), glycerol triacetate (Triacetin) and castor oil were purchased from E-Merck (Schuchardh, Hokenbrunn, Germany). Medium chain triglyceride (Labrafac) and caprylo caproyl macrogol-8-glyceride (Labrasol) were kind gift simples from Gattefosse (Cedex, France). All other chemicals used were of analytical reagent (AR) grade.

3.2. Solubility study

To develop an oral formulation, solubility of drug in oils is important as the ability of nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the 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 and castor oil) and 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 bath (Nirmal International, Delhi, India) for 72 h to reach to 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 (Hogan et al. 2000). Solubility of ramipril in surfactants and co-surfactants were also determined so that the number of surfactants and co-surfactants used in the study can be reduced.

3.3. Construction of phase diagram

On the basis of the solubility study, Sefsol 218, Labrasol and Carbitol were used as oil phase, surfactant and cosurfactant respectively. Based on maximum stability of ramipril, standard buffer solution pH 5 (I.P., 85) was used as an aqueous phase (Shafiq and Shakeel 2008). The relationship between the phase behavior of a mixture and its composition can be captured with construction of a phase diagram (Lawrence and Rees 2000).
Surfactant and co-surfactant (S_{mix}) were mixed in different weight ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1 and 4:1) and the stock of 100 ml of different S_{mix} were prepared. These S_{mix} ratios were chosen in increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams in nanoemulsion formation.
For each phase diagram, oil and specific S_{mix} ratio was mixed thoroughly in different weight ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and S_{mix}, 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 an aqueous titration method. Slow titration with aqueous phase was done to each combination of oil and S_{mix} separately. The amount of aqueous phase added was varied to give an aqueous concentration in the range of 5–95% of total weight at 5% intervals. After every 5% addition of the aqueous phase to the oil and S_{mix} mix, visual observation was made and recorded for easily flowable o/w nanoemulsion (NE).
This was done so that the boundaries of the nanoemulsion and different phases could be properly delineated. The physical state of the nanoemulsion was marked on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the other representing the oil phase and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios (S_{mix} ratio). These observations were made for each S_{mix} ratio separately and for each S_{mix} ratio phase diagram was constructed separately.

3.4. Formulation selection

From each phase diagram constructed, different formulations were selected from the nanoemulsion region so that ramipril could be incorporated into the oil phase; therefore, the following criteria were made for the selection of different formulation from phase diagrams:

- The dose of ramipril varies between 1.5 mg to 20 mg, the frequently prescribed dose is 5 mg for the adult. Therefore, 5 mg was selected for the development of nanoemulsion formulation.
- The oil concentration should be selected in a way that it dissolves the drug (5 mg) easily.
- To check if there is any effect of drug on the phase behavior and nanoemulsion area of the phase diagram.
- From each phase diagram different concentration of oil, which solubilized, was selected at a difference of 5 % (10, 15, 20, 25, 30 and 35 %).
- For each percentage of oil selected, three formulas were taken from the phase diagram:One, which used a minimum concentration of S_{mix} for its nanoemulsion formation.Second, that contained around 5 % extra S_{mix} than the first, so that it is slightly higher than the formula on the line to avoid metastable formulations wherever possible.Third, formula was selected from the middle point of the S_{mix} used for that percentage of oil wherever possible, which was regarded as the reference for the above two formulae.
- For convenience purposes, 1 ml was selected as the dose of the nanoemulsion formulation.

As per saturation solubility studies of ramipril in oil, Sefsol 218, around 200 mg of drug can be solubilized per ml of oil. 5% (0.05 ml) of oil in 1 ml formulation should be able to solubilize 5 mg of ramipril, which is very near to its saturation solubility and can thus even precipitate. Therefore 10% was selected as the least oil concentration to be taken for 1 ml formulation.
The stock solutions of oil at a difference of 5% were prepared in which drug was dissolved in such a way that 5 mg dose is present in each formulation. Formulation (1 ml) was prepared from each phase diagram according to the criteria above. Selected formulations were subjected to different thermodynamic stability and self-nanoemulsification efficiency tests.

3.5. Thermodynamic stability studies

Nanoemulsions are thermodynamically stable systems with no phase separation, creaming or cracking. Care must be taken to ensure that observations are not made on metastable systems. Therefore selected formulations were subjected to thermodynamic stability stress tests as heating cooling cycle, centrifugation and freeze thaw cycle (Shafiq et al. 2007a, b).

3.6. Self-nanoemulsification efficiency test

For oral nanoemulsions the process of dilution by the gastrointestinal (GI) fluids may result in the gradual desorption of surfactant located at the globule interface leading to precipitation of the drug or phase separation of the nanoemulsion making the formulation useless. Thus, a self-nanoemulsification efficiency test was carried out to assess the efficiency of nanoemulsion. The efficiency of self-nanoemulsification of oral nanoemulsion was assessed using a standard USP XXII dissolution apparatus 2 (Shafiq et al. 2007a). Each formulation (1 ml) was added to 500 ml of distilled 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.

The formulations those passed the thermodynamic stability and also self-nanoemulsification efficiency test in Grade A or B were taken for the further studies.

Acknowledgement: The authors are grateful to Ranbaxy Research Laboratory, India and Nikko Chemicals, Japan for providing the gift sample of ramipril base and Sefsol 218 respectively.

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