Formulation Development and Optimization Using Nanoemulsion Technique: A Technical Note

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INTRODUCTION

The design of effective formulations for drugs has long been a major challenge, because drug efficacy can be severely limited by instability or poor solubility in the vehicle. One of the most promising technologies is the nanoemulsion drug delivery system, which is being applied to enhance the solubility and bioavailability of lipophilic drugs. The nanosized droplets leading to an enormous increase in interfacial areas associated with nanoemulsion would influence the transport properties of the drug.[1,2]

Ramipril, 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 6 times the angiotensin-converting enzyme inhibitor activity of ramipril. Ramipril, a lipophilic (log *P* [octanol/water], 3.32), poorly water soluble drug with around 28% to 30% variable oral absorption, was selected as the model drug for the study.

Nanoemulsions are prepared by the spontaneous emulsification method (titration method). They can be prepared simply by blending oil, water, surfactant, and cosurfactant, in the right proportion, with mild agitation. The order of mixing the components is generally considered not to be critical since nanoemulsions are formed spontaneously. Although nanoemulsification is a spontaneous process, the driving forces are small and the time taken for these systems to reach equilibrium can be long.[3] To the best of our knowledge, the aqueous titration method used for constructing the phase diagram and the calculations involved for its construction have not been reported in detail. In addition, the basis of selecting different nanoemulsion or microemulsion formulations from the phase diagrams has not been reported, as hundreds of formulations can be prepared from the nanoemulsion region of the phase diagram. The objective of this technical report is to explain the basis for calculations and construction of pseudoternary phase diagrams and to give an idea for selection of nanoemulsion formulations from the phase diagrams, to avoid metastable formulations in the least possible time.

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MATERIALS AND METHODS

Components

Ramipril base was a gift from Ranbaxy Research Labs (Haryana, India). Propylene glycol monocaprylic ester (Sefsol 218) was a gift from Nikko Chemicals (Tokyo, Japan). The medium-chain triglyceride Labrafac was a gift from Gattefossé (Saint Priest, Cedex, France), and diethylene glycol monoethyl ether (Carbitol) was purchased from Merck Schuchardt (Hohenbrunn, Germany). Polyoxy-35-castor oil (Cremophor EL) was purchased from Sigma Aldrich Inc (St Louis, MO). Isopropyl myristate (IPM), glycerol triacetate (triacetin), castor oil, sodium perchlorate AG, and acetonitrile high-performance liquid chromatography (HPLC) grade were purchased from E Merck (Mumbai, India). Water was obtained from a Milli-Q water purification system (Millipore, Billerica, MA). All other chemicals were of analytical grade.

Solubility Study

The solubility of ramipril in various oils (Sefsol 218, triacetin, IPM, Labrafac, castor oil) and distilled water (Figure 1) was determined by adding an excess amount of drug to 2 mL of selected oils and distilled water separately in 5-mL stopper vials, and mixing using a vortex mixer. The vials were then kept at 25 ± 1.0°C in an isothermal shaker (Nirmal International, Delhi, India) for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and filtered through a 0.45-µm membrane filter. The concentration of ramipril was determined in the oils and in water using HPLC at 210 nm.[4]

Construction of Phase Diagram

Surfactant (Cremophor EL) and cosurfactant (Carbitol) were mixed (Smix) in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1, etc). These Smix ratios were chosen to reflect

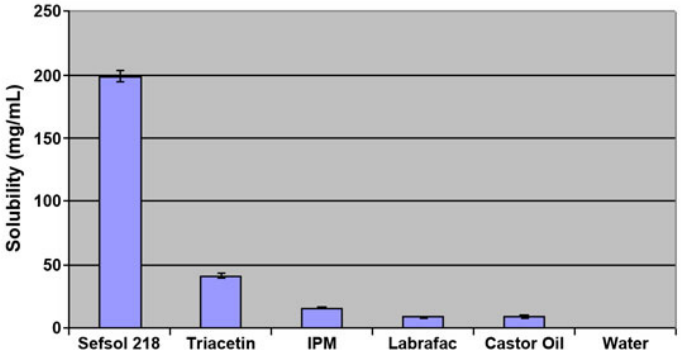

Figure 1. Solubility of ramipril in different oils. IPM indicates isopropyl myristate.

increasing concentrations of cosurfactant with respect to surfactant and increasing concentrations of surfactant with respect to cosurfactant for detailed study of the phase diagrams in the nanoemulsion formation. Sefsol 218 optimized as an oil phase based on the solubility study. For each phase diagram, oil (Sefsol 218) and specific Smix ratio were mixed thoroughly in different volume 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, 1:4, 1:3.5, 1:3, 1:2.33, 1:2, 1:1.5, 1:1, 1:0.66, 1:0.43, 1:0.25, and 1:0.11) were made for the study to delineate the boundaries of phases precisely formed in the phase diagrams.

Pseudoternary phase diagrams were developed using the aqueous titration method. Slow titration with the aqueous phase was performed for each combination of oil and Smix separately. The amount of aqueous phase added was varied to produce a water concentration in the range of 5% to 95% of total volume at around 5% intervals. The calculation for the addition of aqueous phase was done by calculating the percentage of each component of the nanoemulsion present at each 5% addition. For the purpose of explanation, a 1:9 ratio of oil and Smix was taken and the aqueous phase was added using a micropipette at around 5% intervals (Table 1) by mixing on a vortex mixer. The beauty of this system is that the scale-up of the proportions is easy, as the system is thermodynamically stable.

After each 5% addition of the aqueous phase to the oil: Smix mixture, visual observation was made and recorded in Table 2. Through visual observation the following categories were assigned:

1. transparent and easily flowable: oil/water nanoemulsions (Figure 2)
2. transparent gel: nanoemulsion gel
3. milky or cloudy: emulsion (Figure 2)
4. milky gel: emulgel

In a similar manner, calculations for the other ratios of oil and Smix were also done; observations appear in Table 2. The physical state marked in Table 2 was plotted on a pseudo-three-component phase diagram with 1 axis representing the aqueous phase, the second representing the oil phase, and the third representing a mixture of surfactant and cosurfactant at a fixed volume ratio (Figure 3). For each Smix ratio, a separate phase diagram was constructed, and for each phase

Table 1. Calculation for Percentage of Oil, Surfactant, and Water Used in the Construction of Phase Diagram (Oil and Smix Is in the Ratio of 1:9)

Oil µL	Surfactant (Smix) µL	Water* µL	Water Added† µL	Total µL	Oil %	Surfactant (Smix) %	Water %
10	90	10	0	110	9.09	81.82	9.09
10	90	20	10	120	8.33	75.00	16.67
10	90	25	5	125	8.00	72.00	20.00
10	90	35	10	135	7.41	66.67	25.93
10	90	45	10	145	6.90	62.07	31.03
10	90	55	10	155	6.45	58.06	35.48
10	90	65	10	165	6.06	54.55	39.39
10	90	80	15	180	5.56	50.00	44.44
10	90	100	20	200	5.00	45.00	50.00
10	90	120	20	220	4.55	40.91	54.55
10	90	150	30	250	4.00	36.00	60.00
10	90	185	35	285	3.51	31.58	64.91
10	90	235	50	335	2.99	26.87	70.15
10	90	300	65	400	3.00	23.00	75.00
10	90	400	100	500	2.00	18.00	80.00
10	90	550	150	650	2.00	14.00	85.00
10	90	900	350	1000	1.00	9.00	90.00
10	90	2000	1100	2100	0.48	4.29	95.24

*The amount of water is varied to provide a water concentration in the range of 5% to 95% of total volume at around a 5% interval.

†The water added is that added to the previous mixture.

Table 2. Visual Observation During Aqueous Phase Titration for Phase Diagram Construction (Figure 1) Using Smix Ratio 1:1*

	Observation Made After Each Addition of Aqueous Phase																	
Ratio Oil:Smix µL	10 µL	10 µL	5 µL	10 µL	10 µL	10 µL	10 µL	15 µL	20 µL	20 µL	30 µL	35 µL	50 µL	65 µL	100 µL	150 µL	350 µL	1100 µL
(1:9) 10:90	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
(1:4) 20:80	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	E	E
(1:2.33) 30:70	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	E	E	E	E
(1:1.5) 40:60	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	E	E	E	E	E
(1:1) 50:50	NE	NE	NE	NE	NE	NE	NE	NE	E	E	E	E	E	E	E	E	E	E
(1:0.66) 60:40	EG	EG	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
(1:0.43) 70:30	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
(1:0.25) 80:20	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
(1:0.11) 90:10	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
(1:2) 20:40	6 µL NE	5 µL NE	4 µL NE	5 µL NE	6 µL NE	7 µL NE	7 µL NE	10 µL NE	10 µL NE	15 µL NE	15 µL NE	22 µL E	28 µL E	40 µL E	60 µL E	100 µL E	200 µL E	600 µL E
(1:3) 20:60	10 µL NE	4 µL NE	6 µL NE	7 µL NE	8 µL NE	8 µL NE	11 µL NE	12 µL NE	14 µL NE	18 µL NE	22 µL NE	29 µL NE	38 µL NG	53 µL NG	80 µL E	140 µL E	250 µL E	820 µL E
(1:3.5) 20:70	10 µL NE	6 µL NE	7 µL NE	7 µL NE	9 µL NE	10 µL NE	11 µL NE	14 µL NE	16 µL NE	20 µL NE	25 µL NE	33 µL NE	42 µL E	60 µL E	90 µL E	150 µL E	300 µL E	900 µL E
(1:5) 20:100	14 µL NE	8 µL NE	8 µL NE	10 µL NE	13 µL NE	12 µL NE	15 µL NE	20 µL NE	20 µL NE	27 µL NE	33 µL NE	45 µL NE	55 µL NE	80 µL NE	120 µL NE	200 µL NE	420 µL NE	1200 µL NE
(1:6) 20:120	16 µL NE	9 µL NE	10 µL NE	12 µL NE	13 µL NE	16 µL NE	17 µL NE	22 µL NE	25 µL NE	32 µL NE	38 µL NE	50 µL NE	70 µL NE	90 µL NE	140 µL NE	240 µL NE	460 µL NE	1400 µL NE
(1:7) 20:140	18 µL NE	12 µL NE	10 µL NE	14 µL NE	16 µL NE	16 µL NE	21 µL NE	28 µL NE	25 µL NE	36 µL NE	44 µL NE	60 µL NE	75 µL NE	110 µL NE	160 µL NE	260 µL NE	530 µL NE	1600 µL NE
(1:8) 20:160	20 µL NE	12 µL NE	13 µL NE	15 µL NE	18 µL NE	19 µL NE	23 µL NE	27 µL NE	33 µL NE	40 µL NE	50 µL NE	65 µL NE	85 µL NE	120 µL NE	180 µL NE	300 µL NE	600 µL NE	1800 µL NE

*Oil used: Sefsol 218; surfactant used: Cremophor EL; cosurfactant used: Carbitol; Smix ratio: 1:1. NE indicates oil/water nanoemulsion; NG, nanoemulsion gel; EG, emulgel; E, emulsion or phase separation.

diagram Table 2 was used to record the visual observation. Table 2 contains visual observations made for the Smix ratio of 1:1, and the pseudoternary phase diagram (Figure 3) was constructed based on the observations noted in the table. In this figure, only nanoemulsion points are plotted (shaded area), so that there is no overcrowding of the phases in the diagram, as for formulation development only the nanoemulsion area is of interest.

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 used for the selection of different formulations from the phase diagrams:

1. Five milligrams of ramipril was selected as a dose for incorporation into the oil phase.
2. For convenience, 1 mL was selected as the nanoemulsion formulation, so that it could be increased or decreased as per the requirement in the proportions.
3. 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.

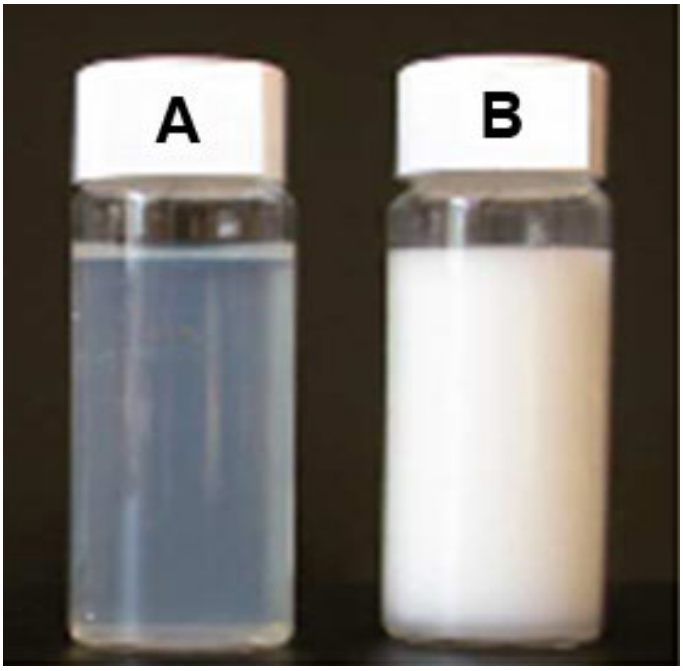

Figure 2. A nanoemulsion (a) and a macroemulsion (b) with droplet diameters of less than 100 nm and more than 1000 nm, respectively.

4. From each phase diagram, different concentrations of oil were selected at a difference of 5% (10%, 15%, 20%, 25%, etc) from the nanoemulsion region.
5. The effect of ramipril on the phase behavior and nanoemulsion area of the phase diagram was checked.
6. For each percentage of oil selected, the formula that used the minimum concentration of Smix for its nanoemulsion formation was selected from the phase diagram.

Different formulations were selected from Figure 3 on the above-based criteria and were subjected to different thermodynamic stability tests.

Thermodynamic Stability Studies

To overcome the problem of metastable formulation, thermodynamic stability tests were performed. Selected formulations were centrifuged at 3500 rpm for 30 minutes. Those formulations that did not show any phase separations were taken for the heating and cooling cycle. Six cycles between refrigerator temperatures of 4°C and 45°C for 48 hours were done. The formulations that were stable at these temperatures were subjected to the freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulations between –21°C and +25°C.

Those formulations that survived thermodynamic stability tests were selected for the further studies. Compositions of these formulations are given in Table 3.

Droplet Size Analysis

The droplet size of the nanoemulsion was determined by photon correlation spectroscopy. The formulation (0.1 mL) was dispersed in 50 mL of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using a Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). Light scattering was monitored at 25°C at a 90° angle (Table 3).

Transmission Electron Microscopy

The morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM). A TOPCON 002B operating at 200 kV capable of point-to-point resolution was used. A combination of bright-field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. To perform the TEM observations, the nanoemulsion formulation was diluted with water (1/100). A drop of the diluted nanoemulsion was directly deposited on the holey film grid and observed after drying (Figure 4).

Viscosity Determination

The viscosity of the formulations (0.5 g) was determined without dilution (Table 3) using a 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 viscosity calculations was Rheocalc V2.6.

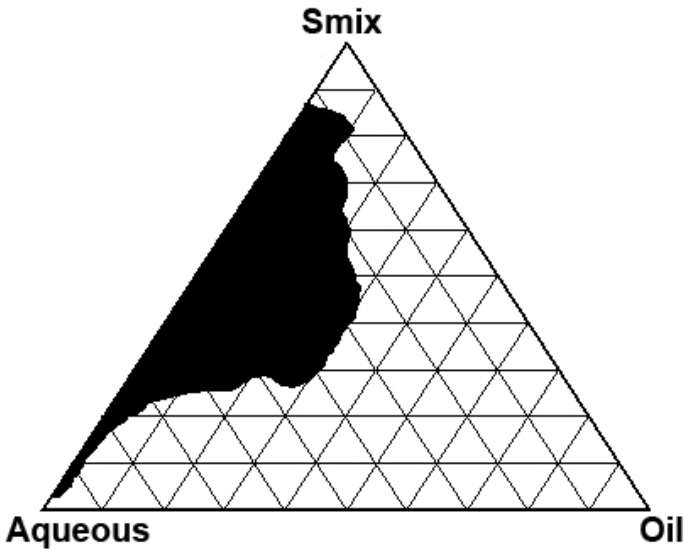

Figure 3. Representative pseudoternary phase diagram of surfactant and cosurfactant (Smix) mixture ratio 1:1, showing oil/water nanoemulsion area (shaded area).

Table 3. Selected Formulations From Figure 3, at a Difference of 5% Vol/Vol of Oil, With Their Droplet Size, Polydispersity, and Mean Viscosity (n = 3)

Percentage Vol/Vol of Different Components in Formulation			Mean Droplet		Mean Viscosity	
Oil*	Smix	Aqueous Phase	Size (nm)	Polydispersity	(cP)	Code
10	26	64	31.6	0.526	117.16 ± 3.01	CF1
15	27	58	32.8	0.180	119.57 ± 2.91	CF2
20	27	53	34.5	0.037	119.28 ± 1.99	CF3
25	25	50	35.0	0.070	120.75 ± 2.18	CF4
30	30	40	36.7	0.111	122.15 ± 3.21	CF5

*Oil phase is loaded with drug (ramipril).

Results and Discussion

The important criterion for selection of components is their pharmaceutical acceptability. It has been demonstrated that only very specific pharmaceutical excipient combinations lead to efficient nanoemulsion formulations.[2,5] The solubility of the drug in oils is most important, as the ability of the nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase. If the surfactant or cosurfactant is contributing to drug solubilization, there could be a risk of precipitation, particularly when oral or parenteral nanoemulsion is the goal.[2] The solubility of ramipril in different oils was determined because for drug substances with a log P value around 3 to 5, there is no clear trend regarding the type of oil that will cause the highest increase in solubility.[6] Among the oils, the solubility of ramipril was found to be highest in Sefsol 218 (199.33 ± 4.04 mg/mL), while in water it was 0.09 ± 0.01 mg/mL (Figure 1). Thus, Sefsol 218 was selected as the oil phase for the development of the formulation.

The surfactant chosen must be able to lower the interfacial tension to a very small value to aid the dispersion process during the preparation of the nanoemulsion, provide a flexible film that can readily deform around droplets, and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region for the desired nanoemulsion type (ie, oil/water, water/oil, or bicontinuous).[1-3,5] In the present study, when cosurfactant was added along with surfactant in an equal ratio (Smix ratio 1:1), it was observed in the phase diagram (Figure 3) that 5% oil could be solubilized by 22% of Smix and 10% to 30% oil could be solubilized by just increasing Smix from 26% to 30% vol/vol. 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 leads to an increase in the fluidity of the interface to take up the different curvatures required to form nanoemulsions over a wide range of compositions.[2,7]

The dose of ramipril varies between 2.5 mg and 20 mg; a frequently prescribed adult dose is 5 mg. Therefore, for the present study, a 5-mg dose was selected for the development of the nanoemulsion formulation. No change was found in the phase behavior of the pseudoternary phase diagram when ramipril was included in the formulation, which may be due to the fact that the formation and stability of nanoemulsions consisting of non-ionic surfactants are not affected by the change in pH or ionic strength.[1,2,8,9]

Hundreds of formulations can be prepared from the nanoemulsion region of the phase diagram (Figure 3). While going through pseudoternary phase diagrams, oil could be solubilized up to the extent of 30% vol/vol. Therefore, from each phase diagram a different concentration of oil that formed a nanoemulsion was selected at 5% intervals (10%, 15%, 20%, 25%, and 30%) so that the largest number of formulations could be selected covering the nanoemulsion area of the phase diagram. It is well reported that large amounts of surfactants, particularly ionic surfactants, cause irritation, so for drug delivery, nonionic surfactants are preferred in as low a concentration as possible.[2] Therefore, selection of formulations was based on the criterion of their being a minimum concentration of Smix used in the formulation. For each percentage of oil selected, only those formulations that

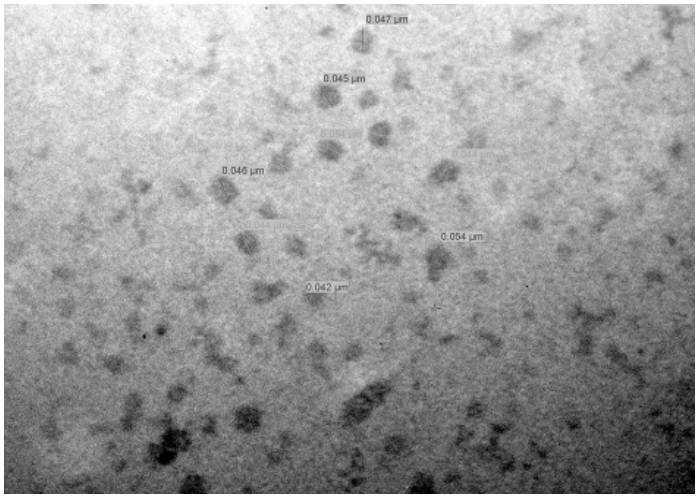

Figure 4. Transmission electron microscopic positive image of ramipril nanoemulsion showing size of some oil droplets.

used a minimum concentration of Smix were taken from the phase diagram (Table 3).

Constructing phase diagrams is time-consuming, particularly when the aim is to accurately delineate a phase boundary, as the time taken for the system to equilibrate can be greatly increased as the phase boundary is approached.[2] Care must be taken to ensure that observations are not made on metastable systems. Clearly, however, time constraints impose a physical limit on the length of time systems can be left to equilibrate, and consequently the elimination of metastable states can be difficult to ensure in practice.[1,2,10] To overcome this problem of metastable formation, thermodynamic stability tests were performed. The formulations selected were subjected to different stress tests, such as centrifugation, heating-cooling cycle, and freeze-thaw cycle tests. If the nanoemulsions are stable over these conditions, metastable formulations are thus avoided and frequent tests need not be performed during storage, unless chemical reactions occur (eg, oxidation, pH variations) that change the nature of the components and hence of the nanoemulsion. The formulations that survived thermodynamic stability tests were subjected to further characterization, such as droplet size, viscosity determination, and TEM.

The droplet size analysis of the selected formulations showed that the size increased with the increase in the concentration of oil in the formulations CF1 to CF5 (Table 3). This may have been due to the increase in the oil concentration from 10% to 30% vol/vol, although the difference in the droplet size between the formulations was not statistically significant ($P > .05$). The polydispersity was at a minimum in the case of CF3, which contained 20% oil, suggesting uniformity of droplet size, 34.5 nm, in the formulation. The droplets in the nanoemulsion appear dark, and the surroundings are bright (Figure 4); a "positive" image was seen using TEM. Some droplet sizes were measured using TEM, as it is capable of point-to-point resolution. The droplet size was in agreement with the results obtained from droplet size analysis using the Zetasizer.

The viscosity of the selected formulations was determined. The values are shown in Table 3. Formulation CF1 had the lowest viscosity, perhaps because of its lower oil content. The difference in viscosities between the formulations was not significant ($P \geq 0.05$), but it can be observed that the viscosity of the nanoemulsion formulations was very low as expected. The nanosized droplets leading to enormous interfacial areas, thereby enhancing the solubility of a poorly soluble drug, would influence the transport properties of the drug. The release study of the system based on the route of administration of the formulation is performed. Nanoemulsion, a multipurpose drug delivery system, can thus be used to deliver drugs by oral, topical, parenteral, and many other routes.

Summary and Conclusion

Ramipril nanoemulsion formulations were successfully prepared by the spontaneous emulsification method (titration method). Sefsol 218 was selected as the oil phase for the development of the formulation on the basis of the solubility studies. The differences in the droplet size between the formulations selected from the phase diagram was not statistically significant, although the polydispersity was at a minimum for the formulation containing 20% oil, 27% Smix, and 53% vol/vol aqueous phase. The droplet size was found to be 34.5 nm. Therefore, nanoemulsion, a multipurpose technology, can be exploited in drug delivery for poorly soluble drugs. 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. This technical note explains the basis for calculation and construction of pseudoternary phase diagrams and, most important, explains selection of the formulations from the phase diagrams to avoid metastable formulations having minimum surfactant concentration in the least possible time.

References

1. Eccleston J. Microemulsions. In: Swarbrick J, Boylan JC, eds. *Encyclopedia of Pharmaceutical Technology.* vol. 9. New York, NY: Marcel Dekker; 1994:375–421.

2. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev.* 2000;45:89–121.

3. Rosano HL, Cavello JL, Lyons GB. Mechanism of formation of six microemulsion systems. In: Rosano HL, Clausse M, eds. *Microemulsion Systems.* New York, NY: Marcel Dekker; 1987:259–257.

4. Belal F, Al-Zaagi IA, Gadkariem MA, Abounassif MA. A stability-indicating LC method for the simultaneous determination of ramipril and hydrochloride in dosage forms. *J Pharm Biomed Anal.* 2001;24: 335–342.

5. Gursoy RN, Benita S. Self-emulsifying drug delivery systems for improved oral delivery of lipophilic drugs. *Biomed Pharmacother.* 2004;58:173–182.

6. Grove M, Pedersen GP, Nielsen JL, Mullertz A. Bioavailability of seocalcitol I: relating solubility in biorelevant media with oral bioavailability in rats—effect of medium and long chain triglycerides. *J Pharm Sci.* 2005;94:1830–1838.

7. Warisnoicharoen W, Lansley AB, Lawrence MJ. Light scattering investigations on dilute non-ionic oil-in-water microemulsions. *AAPS PharmSci.* 2000;2:Article 12.

8. Constantinides PP, Lancaster CM, Marcello J, et al. Enhanced intestinal absorption of an RGD peptide from water-in-oil microemulsions of different composition and particle size. *J Control Release.* 1995;34:109–116.

9. Ghosh PK, Murthy RSR. Microemulsions: a potential drug delivery system. *Curr Drug Deliv.* 2006;3:167–180.

10. Pouton CW. Formulation of self-emulsifying drug delivery systems. *Adv Drug Deliv Rev.* 1997;25:47–58.