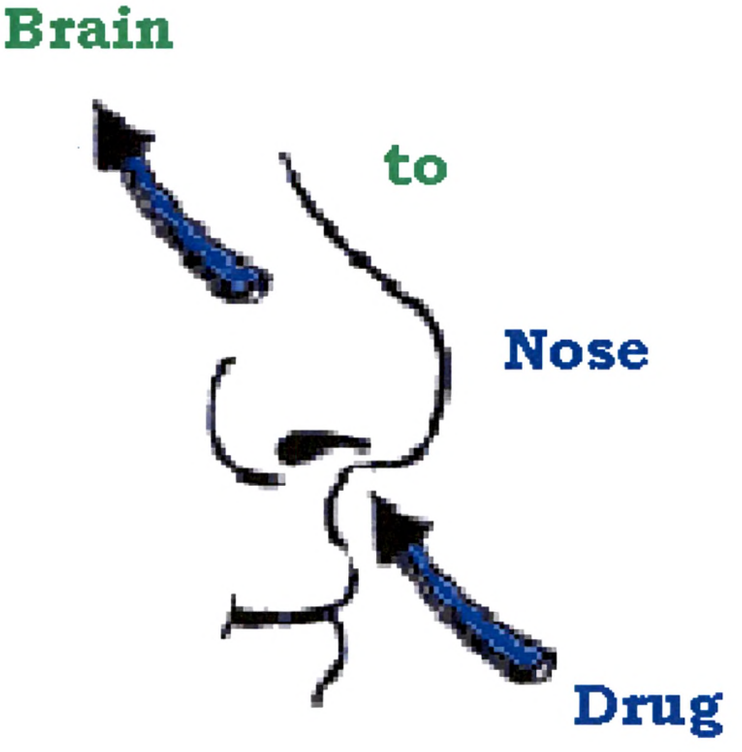

CHAPTER 4

PREPARATION, CHARACTERIZATION, OPTIMIZATION AND STABILITY STUDIES OF MICROEMULSIONS

Microemulsions (ME) are thermodynamically stable systems which can be broadly classified into three major categories (1) oil-in-water (o/w) ME, (2) water-in-oil (w/o) ME, and (3) bicontinuous ME (Shinoda et al. 1991; Shinoda and Friberg 1975). Typical ME can be formulated which is usually constituting four phases viz. (1) aqueous phase (AQ component) which could be water (hydrous) or anhydrous in nature, (2) Oil phase (O component), (3) surfactant (S component), and (4) co-surfactant (CoS component) (Shinoda and Kunieda 1973). Regardless of the type of aforesaid type of ME systems, ME can be formulated easily by mixing the oil component with surfactant and co-surfactant components. Aqueous components can be added gradually to the mixture of oil containing surfactant and co-surfactant components. Since, the ME are thermodynamically stable systems; it undergoes spontaneous formation facilitated as a result of micelle formation without input of external energy into the system (Lawrence and Rees 2000).

Microemulsion formulation techniques have been reported by many researchers and can be sighted from various literatures (Skodvin et al. 1993). These techniques mainly include (1) Titration method, and (2) pseudo-ternary phase diagram construction (Lawrence and Rees 2000).

Titration method is most commonly employed simplest approach particularly when the aim is to accurately delineate the phase boundaries. In titration method, a series of pseudo binary component systems are formed which are titrated using the third component, evaluating the mixture after each addition. Most commonly the third component is the aqueous phase however, surfactants mixture or oil phase can also be employed. Titrating binary phase with the third component will yield an optically clear system from which usually the ratio and concentrations of individual components are derived by back calculation method. Heat and sonication are often employed tools to speed up the process. However, care must be exercised especially when the phase boundary is approached as the time taken may greatly increase for system to equilibrate. Utmost care should be taken to ensure that not only the temperature is accurately and precisely controlled but also that the optical observations for clarity are not made on metastable systems. The advantages associated with titration techniques are (1) rapid, (2) reasonably accurate and precise, and (3) economical as large number of observations can be made using limited excipients and drugs. The major disadvantage with titration technique is that it can provide true picture

of the phase boundaries but the systems existing within the periphery can't be taken in isolated manner for further studies such as characterization (Lawrence and Rees 2000).

Pseudo-ternary phase diagram is a very useful and important tool to study the phase behavior. Pseudo-ternary phase diagram can be represented in a triangular format (triangle) which has three coordinates. Each coordinate represents one component of ME system. A typical pseudo-ternary phase diagram illustrating the different phases on respective coordinates is shown in Figure 4.1. As seen from the Figure 4.1, each coordinate is representing one phase present in the ME system viz. (1) Oil phase (O component), (2) Surfactant: Co-surfactant phase (S: CoS component), and (3) Aqueous phase (AQ component). Each coordinate also represents 0 to 100% concentration of each of the phases in the increment of 10%. In case where four or more components are investigated to formulate ME system, pseudo-ternary phase diagram is used wherein each corner typically represents binary mixture of two components such as surfactant/co-surfactant, water/drug, or oil/drug. Phase diagram is an imperative tool to comprehensively study the ME system and its phase behavior although construction phase diagram is highly time consuming exercise. In addition to that, phase diagram represents 36 ME points hence, for each ratio or a ME system, a number of experiments including excipients and drug are required to expansively study the phase behavior (Prince 1967). However, as a conservative approach, it is a traditional scientific practice to appropriately blend titration technique with phase diagram approach together in order to save time and to make it commercially viable option. In this investigation, an approach derived on the basis of titration technique followed by construction of phase diagram was used. The experiments conducted are represented in the form of two-dimensional phase diagram. Microemulsion has four basic components; oil phase (O component), surfactant (S component), Co-surfactant (CoS component) and aqueous phase (AQ component). ME is represented by a four dimensional point.

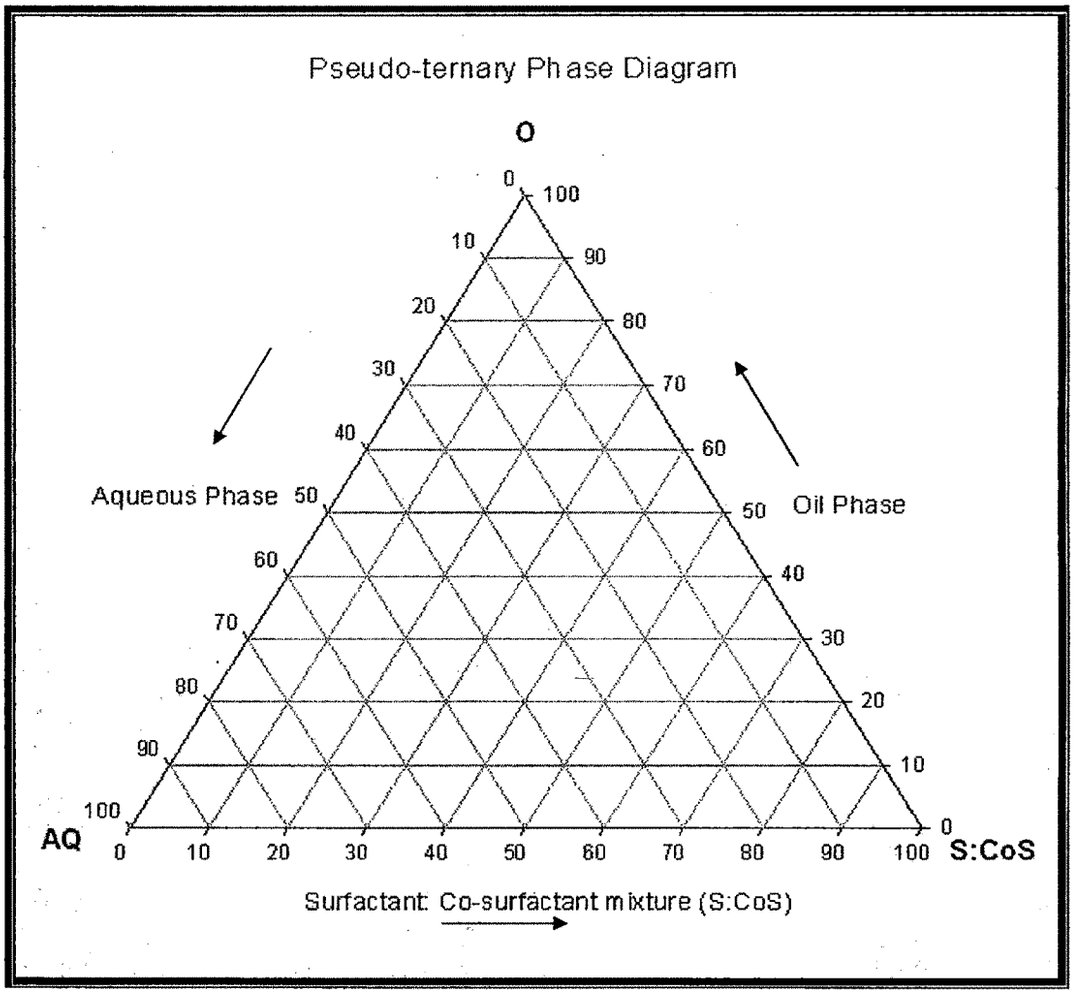

Figure 4.1 Pseudo-ternary phase diagram representing the three phases and various ME points where three phases intercepts.

4.1 Preparation of Microemulsions

4.1.1 Tacrine Microemulsions:

Tacrine Microemulsions – TME (system 1, TME 1) were prepared by titration method using Labrafil M 1944 CS® as an oil phase (O), Cremophor RH 40® as a surfactant (S), and Transcutol P® as a co-surfactant (CoS), and distilled water as an aqueous phase (AQ). Tacrine (33 mg/mL) was dissolved in oil phase containing surfactant and co-surfactant at room temperature with continuous stirring. To the resultant mixture distilled water is added gradually with continuous stirring. Similarly, another set of TME (system 2, TME 2) were prepared using Labrafil M 1944 CS® as oil phase, Cremophor EL® as surfactant, Transcutol P® as co-surfactant and distilled water as an aqueous phase. Tacrine (33 mg/mL) was dissolved in oil phase containing surfactant and co-surfactant at room temperature with continuous stirring. To the resultant mixture distilled water is added gradually with continuous stirring. TME system 3 (TME 3) was prepared by replacing oil phase of TME 1 with Labrafac CC® and TME system 4 (TME 4) was prepared by replacing oil phase of TME 2 with Labrafac CC®. The process was identical as TME 1 and TME 2. The excipient profile for TME system 1, 2, 3 and 4 is shown in Table 4.1.

For optimization of ME composition distilled water was added with stirring to the mixture of oil and surfactant / co-surfactant (at different mass ratios viz. 1:1, 2:1 and 3:1) containing Tacrine. The compositions which are optically clear have been evaluated further by constructing phase diagrams. Visually clear and transparent ME were considered as acceptable. Clarity was further confirmed by measuring percentage transmittance at 630 nm wavelength (Shimadzu UV-1601, Japan) against water as blank (Roland et al. 2003). Microemulsions having transmittance value greater than 99 % were considered as clear. The concentrations of various phases which yielded clear ME are plotted as two dimensional pseudo ternary phase diagram in Figure 4.2 (TME 1), Figure 4.3 (TME 2), Figure 4.4 (TME 3), and Figure 4.5 (TME 4) respectively, to obtain ME region. The concentrations of O, S/ CoS, AQ phase and S: CoS ratios of the clear TME compositions of selected batches were recorded in Table 4.2 (TME 1), Table 4.3 (TME 2), Table 4.4 (TME 3) and Table 4.5 (TME 4) respectively.

Table 4.1 Excipient profiles for four different systems of tacrine microemulsions

Ingredients	System 1	System 2	System 3	System 4
Tacrine	√	√	√	√
Labrafil M 1994 CS® (O)	√	√	☒	☒
Labrafac CC® (O)	☒	☒	√	√
Cremophor RH 40® (S)	√	☒	√	☒
Cremophor EL® (S)	☒	√	☒	√
Transcutol P® (CoS)	√	√	√	√
Water (AQ)	√	√	√	√

S: CoS – 1:1, 2:1 and 3:1 for System 1, 2, 3 and System 4.

√ Ingredients used ☒ Ingredients not used

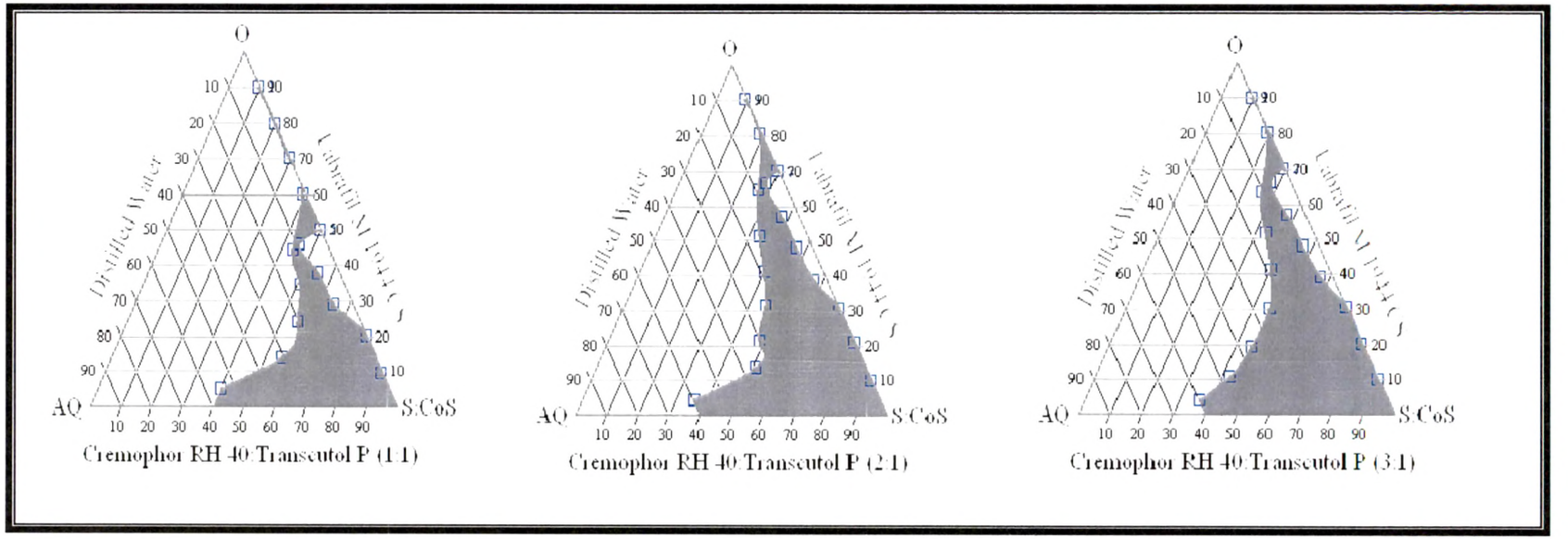

Figure 4.2 Pseudo-ternary phase diagrams for TME 1 (System 1) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

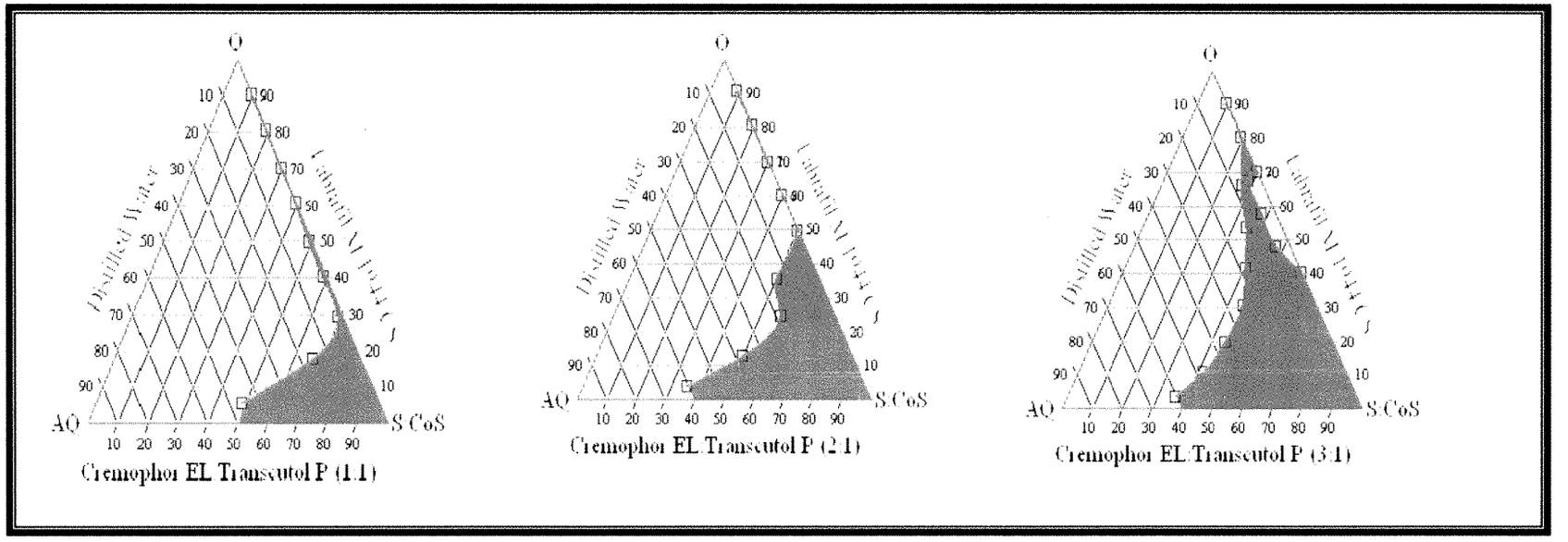

Figure 4.3 Pseudo-ternary phase diagrams for TME 2 (System 2) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

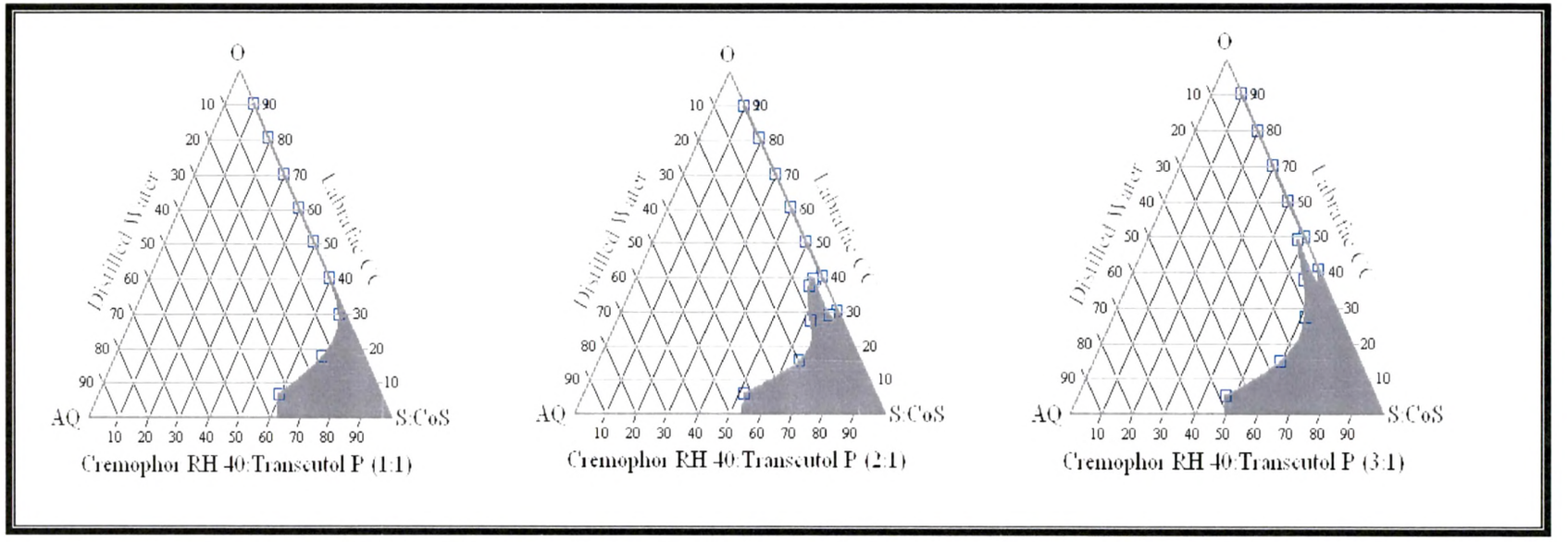

Figure 4.4 Pseudo-ternary phase diagrams for TME 3 (System 3) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

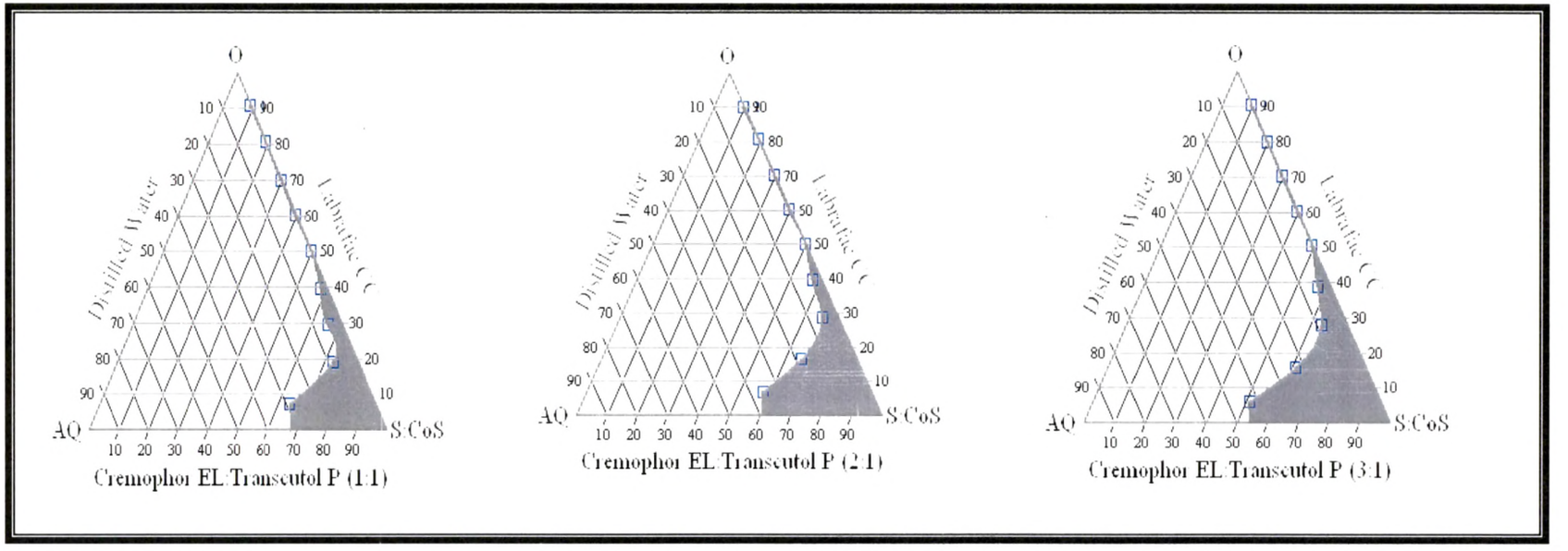

Figure 4.5 Pseudo-ternary phase diagrams for TME 4 (System 4) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

Table 4.2 Compositions and characterization of tacrine microemulsions system 1 (TME 1)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
TME 1 (S:CoS ratio 3:1)	01	10.00	37.50	12.50	40.00	23.45 ± 0.30	-15.32 ± 0.46	99.71 ± 0.36
	02	10.00	41.25	13.75	35.00	25.73 ± 0.24	-18.97 ± 0.82	99.58 ± 0.16
	03	10.00	45.00	15.00	30.00	30.35 ± 0.55	-13.22 ± 0.20	99.34 ± 0.35
	04	15.00	37.50	12.50	35.00	22.42 ± 0.21	-16.56 ± 0.28	99.69 ± 0.24
	05	**15.00**	**41.25**	**13.75**	**30.00**	**23.50 ± 0.40**	**-20.50 ± 0.51**	**99.78 ± 0.42**
	06	15.00	45.00	15.00	25.00	29.35 ± 0.57	-15.92 ± 0.36	99.14 ± 0.33
	07	20.00	37.50	12.50	30.00	34.80 ± 0.65	-15.40 ± 0.33	99.08 ± 0.62
	08	20.00	41.25	13.75	25.00	35.87 ± 0.47	-19.35 ± 0.52	99.31 ± 0.53
	09	20.00	45.00	15.00	20.00	39.23 ± 1.08	-14.23 ± 0.47	99.16 ± 0.40

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Labrafil M 1944 CS®), S for surfactant (Crempohor RH 40®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Distilled Water). The TME formulations contain tacrine – 33 mg/mL.

Table 4.3 Compositions and characterization of tacrine microemulsions system 2 (TME 2)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
TME 2 (S:CoS ratio 3:1)	01	10.00	37.50	12.50	40.00	22.51 ± 0.40	-13.67 ± 0.50	99.80 ± 0.68
	02	10.00	41.25	13.75	35.00	23.70 ± 0.37	-15.87 ± 0.65	99.24 ± 0.59
	03	10.00	45.00	15.00	30.00	26.23 ± 0.82	-12.72 ± 0.78	99.10 ± 0.67
	04	15.00	37.50	12.50	35.00	23.33 ± 0.21	-14.96 ± 0.60	99.54 ± 0.34
	05	**15.00**	**41.25**	**13.75**	**30.00**	**24.00 ± 0.87**	**-19.37 ± 1.27**	**99.12 ± 0.25**
	06	15.00	45.00	15.00	25.00	26.97 ± 0.52	-16.98 ± 0.66	99.46 ± 0.41
	07	20.00	37.50	12.50	30.00	25.03 ± 0.29	-13.48 ± 0.55	99.05 ± 0.38
	08	20.00	41.25	13.75	25.00	28.37 ± 0.35	-15.27 ± 0.72	99.11 ± 0.47
	09	20.00	45.00	15.00	20.00	29.40 ± 0.30	-12.90 ± 0.35	99.20 ± 0.50

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Labrafil M 1944 CS®), S for surfactant (Crempohor EL®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Distilled Water). The TME formulations contain tacrine – 33 mg/mL.

Table 4.4 Compositions and characterization of tacrine microemulsions system (TME 3)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
TME 3 (S:CoS ratio 3:1)	01	10.00	45.00	15.00	30.00	32.35 ± 1.30	-12.28 ± 0.30	99.20 ± 0.40
	02	10.00	48.75	16.25	25.00	38.43 ± 2.12	-14.56 ± 0.34	99.55 ± 0.35
	03	10.00	52.50	17.50	20.00	55.45 ± 0.82	-10.65 ± 0.57	99.42 ± 0.22
	04	15.00	45.00	15.00	25.00	227.56 ± 1.12	-9.84 ± 0.45	97.23 ± 0.64
	05	**15.00**	**48.75**	**16.25**	**20.00**	**44.00 ± 0.95**	**-18.95 ± 0.68**	**99.25 ± 0.40**
	06	15.00	52.50	17.50	15.00	66.56 ± 0.40	-12.56 ± 0.35	99.16 ± 0.64
	07	20.00	45.00	15.00	20.00	425.03 ± 4.40	-4.36 ± 0.45	92.45 ± 2.30
	08	20.00	48.75	16.25	15.00	138.25 ± 1.45	-8.58 ± 0.37	97.82 ± 0.87
	09	20.00	52.50	17.50	10.00	98.30 ± 1.20	-10.50 ± 0.40	97.75 ± 0.70

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Labrafac CC®), S for surfactant (Crempohor RH 40®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Distilled Water). The TME formulations contain tacrine – 33 mg/mL.

Table 4.5 Compositions and characterization of tacrine microemulsions system 4 (TME 4)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
TME 4 (S:CoS ratio 3:1)	01	10.00	45.00	15.00	30.00	35.67 ± 0.87	-12.90 ± 0.80	99.80 ± 0.68
	02	10.00	48.75	16.25	25.00	46.68 ± 0.59	-15.82 ± 0.53	99.24 ± 0.59
	03	10.00	52.50	17.50	20.00	65.20 ± 0.70	-14.23 ± 0.62	99.10 ± 0.67
	04	15.00	45.00	15.00	25.00	323.50 ± 3.30	-7.45 ± 0.55	93.54 ± 0.34
	05	**15.00**	**48.75**	**16.25**	**20.00**	**42.36 ± 1.27**	**-19.62 ± 0.85**	**99.12 ± 0.25**
	06	15.00	52.50	17.50	15.00	72.42 ± 1.46	-13.32 ± 0.43	99.46 ± 0.41
	07	20.00	45.00	15.00	20.00	496.34 ± 4.35	-3.58 ± 0.43	91.83 ± 3.54
	08	20.00	48.75	16.25	15.00	285.75 ± 3.25	-4.45 ± 0.65	94.23 ± 2.37
	09	20.00	52.50	17.50	10.00	89.35 ± 1.40	-10.80 ± 0.85	99.10 ± 0.85

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Labrafac CC®), S for surfactant (Crempohor EL®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Distilled Water). The TME formulations contain tacrine – 33 mg/mL.

4.1.2 Tacrine mucoadhesive microemulsions:

Tacrine mucoadhesive microemulsions (TMME) were prepared by addition of mucoadhesive agents such as chitosan or Carbopol 934 P to optically clear TME (Behl et al. 1998; Ugwoke et al. 2001; Sinsawat et al. 2003; Alpar et al. 2005). TME were prepared as described under section 4.1.1. and chitosan was added in a concentration of 0.25% w/w, 0.50% w/w and 1.0% w/w with continuous stirring. The resultant TMME was stirred for 30 min and allowed to hydrate for 12 h. Similarly another set of TMME were prepared by adding Carbopol 934 P in a concentration of 0.25% w/w, 0.50% w/w and 1.0% w/w to TME, with continuous stirring. The resultant TMME was stirred for 30 min and allowed to hydrate for 12 h.

4.1.3 Donepezil microemulsions:

Donepezil microemulsions – DME (system 1, DME1) were prepared by titration method using Captex 355® as an oil phase (O), Tween 80 as surfactant (S), Capmul MCM as co-surfactant (CoS) and distilled water as an aqueous phase (AQ). Donepezil (16.67 mg/mL) was dissolved in oil phase containing surfactant and co-surfactant at room temperature with continuous stirring. To the resultant mixture distilled water is added gradually with continuous stirring. DME system 2 (DME 2) was prepared by using Labrafil M 1944 CS® as oil phase, Cremophor RH 40® as surfactant, Transcutol P® as co-surfactant and distilled water as an aqueous phase. Donepezil (16.67 mg/mL) was dissolved in oil phase containing surfactant and co-surfactant at room temperature with continuous stirring. Similarly, another set of DME (system 3, DME 3) was prepared replacing oil phase of DME 2 with Labrafac CC®. The method of preparation was identical as DME 2. The excipient profile for DME system 1, 2, 3 and 4 is shown in Table 4.6.

For optimization of ME composition distilled water was added with stirring to the mixture of oil and surfactant / co-surfactant (at different mass ratios viz. 1:1, 2:1 and 3:1) containing donepezil. The compositions which are optically clear have been evaluated further by constructing phase diagrams. Visually clear and transparent ME were considered as acceptable. Clarity was further confirmed by measuring percentage transmittance at 630 nm wavelength (Shimadzu UV-1601, Japan) against water as blank (Roland et al. 2003). Microemulsions having transmittance value greater than 99 % were considered as clear. The concentrations of various phases which yielded clear ME are as plotted two dimensional pseudo ternary phase diagram in Figure 4.6 (DME 1), Figure 4.7 (DME 2), and Figure 4.8 (DME 3) respectively, to obtain ME region. The concentrations

of O, S/ CoS, AQ phase, and S: CoS ratios of the clear DME compositions of selected batches were recorded in Table 4.7 (DME 1), Table 4.8 (DME 2), and Table 4.9 (DME 3) respectively.

Table 4.6 Excipient profiles for four different systems of donepezil microemulsions

Ingredients	System 1	System 2	System 3
Donepezil	√	√	√
Captex 355® (O)	√	☒	☒
Tween 80 (S)	√	☒	☒
Capmul MCM® (CoS)	√	☒	☒
Labrafil M 1994 CS® (O)	☒	√	☒
Labrafac CC® (O)	☒	☒	√
Cremophor RH 40® (S)	☒	√	√
Transcutol P® (CoS)	☒	√	√
Water (AQ)	√	√	√

S: CoS – 1:1, 2:1 and 3:1 for System 1, 2, 3 and System 4.

√ Ingredients used ☒ Ingredients not used

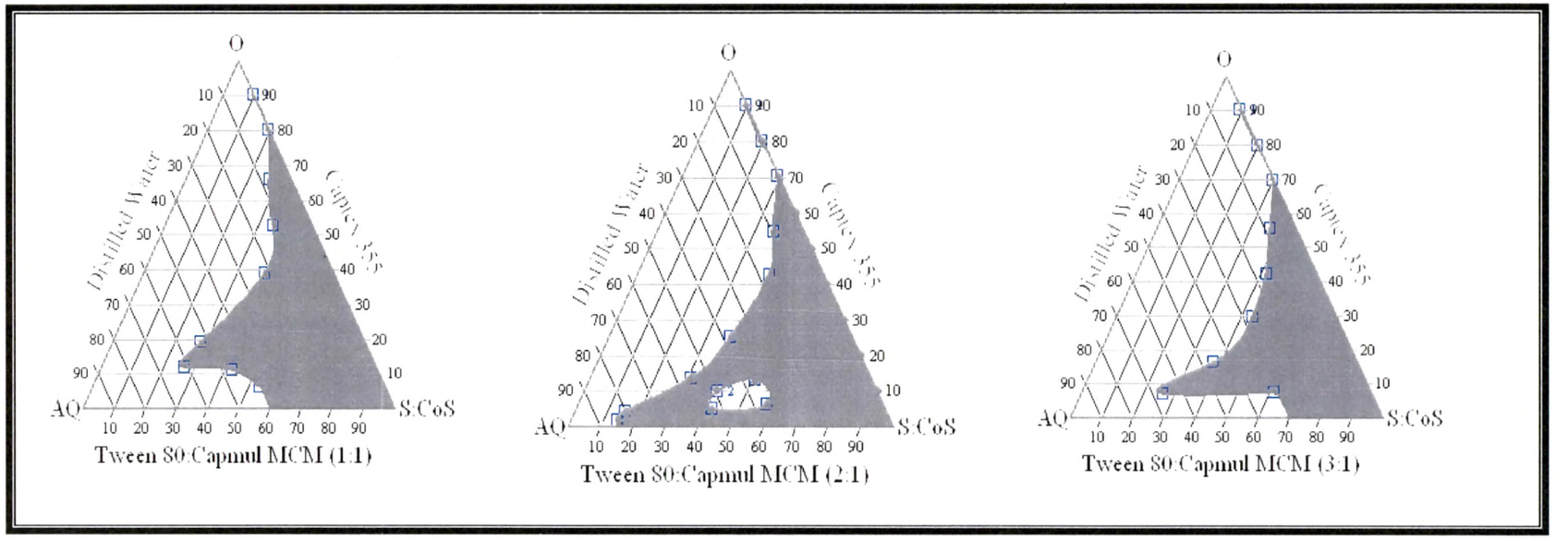

Figure 4.6 Pseudo-ternary phase diagrams for DME 1 (System 1) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

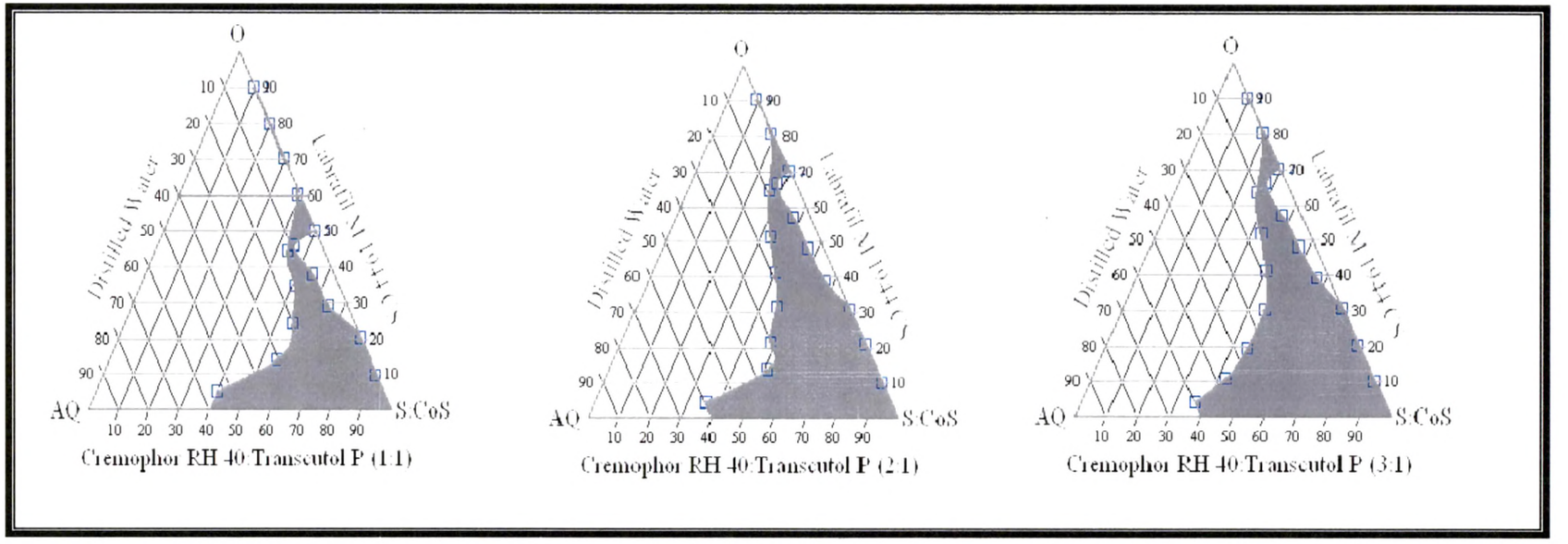

Figure 4.7 Pseudo-ternary phase diagrams for DME 2 (System 2) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

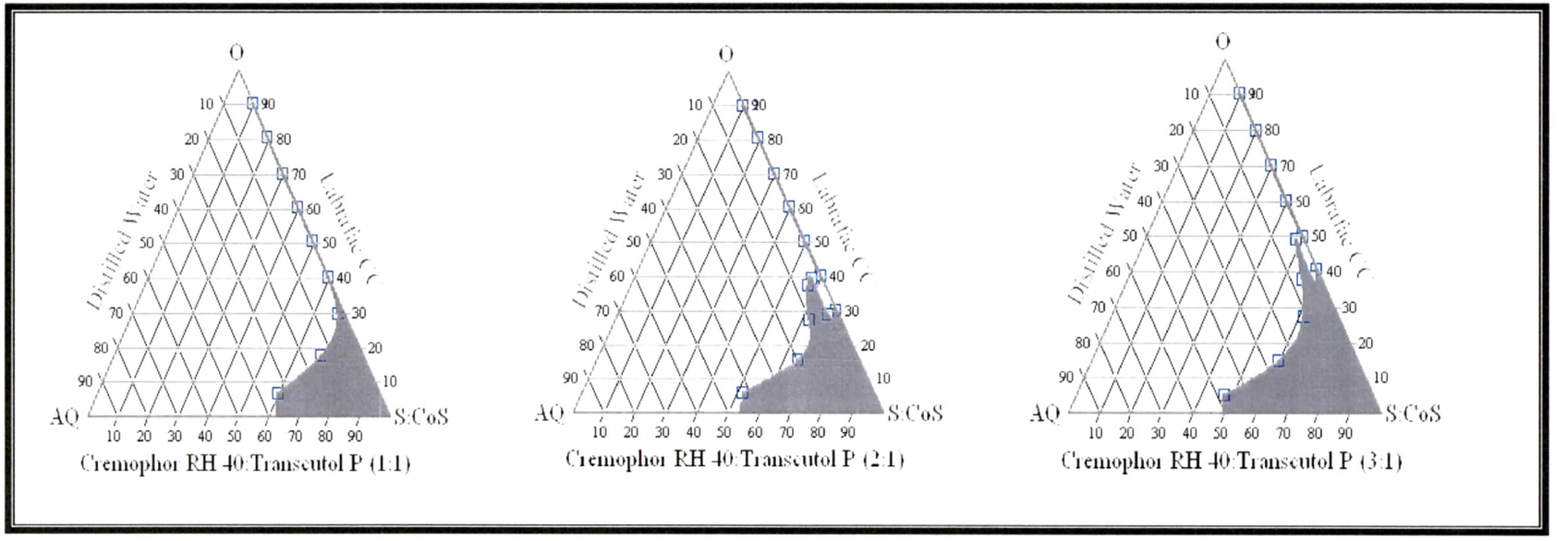

Figure 4.8 Pseudo-ternary phase diagrams for DME 3 (System 3) showing ME regions (Shaded) at S: CoS ratio 1:1, 2:1 and 3:1.

Table 4.7 Compositions and characterization of donepezil microemulsions system 1 (DME 1)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
DME 1 (S:CoS ratio 1:1)	01	20.00	20.00	20.00	40.00	28.34 ± 1.30	-19.15 ± 1.02	99.43 ± 0.58
	02	20.00	22.50	22.50	35.00	33.08 ± 3.02	-20.24 ± 1.14	99.62 ± 0.77
	03	20.00	25.00	25.00	30.00	45.58 ± 3.71	-15.43 ± 0.64	99.57 ± 0.57
	04	**25.00**	**20.00**	**20.00**	**35.00**	**39.75 ± 2.57**	**-20.78 ± 1.26**	**99.23 ± 0.33**
	05	**25.00**	**22.50**	**22.50**	**30.00**	**41.58 ± 1.51**	**-23.49 ± 1.48**	**99.44 ± 0.29**
	06	25.00	25.00	25.00	25.00	79.08 ± 2.08	-16.77 ± 0.65	99.21 ± 0.56
	07	30.00	20.00	20.00	30.00	50.42 ± 1.68	-17.65 ± 0.72	99.26 ± 0.49
	08	30.00	22.50	22.50	25.00	64.08 ± 3.15	-21.57 ± 1.06	99.04 ± 0.31
	09	30.00	25.00	25.00	20.00	88.66 ± 4.18	-14.34 ± 0.88	99.08 ± 0.83

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Captex 355®), S for surfactant (Tween 80), CoS for co-surfactant (Capmul MCM®) and AQ is denoted for aqueous phase (Distilled Water). The DME formulations contain donepezil – 16.67 mg/mL.

Table 4.8 Compositions and characterization of donepezil microemulsions system (DME 2)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
DME 2 (S:CoS ratio 3:1)	01	10.00	37.50	12.50	40.00	23.45 ± 0.30	-15.32 ± 0.46	99.71 ± 0.36
	02	10.00	41.25	13.75	35.00	25.73 ± 0.24	-18.97 ± 0.82	99.58 ± 0.16
	03	10.00	45.00	15.00	30.00	30.35 ± 0.55	-13.22 ± 0.20	99.34 ± 0.35
	04	15.00	37.50	12.50	35.00	22.42 ± 0.21	-16.56 ± 0.28	99.69 ± 0.24
	05	**15.00**	**41.25**	**13.75**	**30.00**	**23.50 ± 0.40**	**-20.50 ± 0.51**	**99.78 ± 0.42**
	06	15.00	45.00	15.00	25.00	29.35 ± 0.57	-15.92 ± 0.36	99.14 ± 0.33
	07	20.00	37.50	12.50	30.00	34.80 ± 0.65	-15.40 ± 0.33	99.08 ± 0.62
	08	20.00	41.25	13.75	25.00	35.87 ± 0.47	-19.35 ± 0.52	99.31 ± 0.53
	09	20.00	45.00	15.00	20.00	39.23 ± 1.08	-14.23 ± 0.47	99.16 ± 0.40

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Labrafil M 1944 CS®), S for surfactant (Crempohor RH 40®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Distilled Water). The DME formulations contain donepezil – 16.67 mg/mL.

Table 4.9 Compositions and characterization of donepezil microemulsions system 3 (DME 3)

System	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Transmittance (%) ± SD
DME 3 (S:CoS ratio 3:1)	01	10.00	45.00	15.00	30.00	32.35 ± 1.30	-12.28 ± 0.30	99.20 ± 0.40
	02	10.00	48.75	16.25	25.00	38.43 ± 2.12	-14.56 ± 0.34	99.55 ± 0.35
	03	10.00	52.50	17.50	20.00	55.45 ± 0.82	-10.65 ± 0.57	99.42 ± 0.22
	04	15.00	45.00	15.00	25.00	227.56 ± 1.12	-9.84 ± 0.45	97.23 ± 0.64
	05	**15.00**	**48.75**	**16.25**	**20.00**	**44.00 ± 0.95**	**-18.95 ± 0.68**	**99.25 ± 0.40**
	06	15.00	52.50	17.50	15.00	66.56 ± 0.40	-12.56 ± 0.35	99.16 ± 0.64
	07	20.00	45.00	15.00	20.00	425.03 ± 4.40	-4.36 ± 0.45	92.45 ± 2.30
	08	20.00	48.75	16.25	15.00	138.25 ± 1.45	-8.58 ± 0.37	97.82 ± 0.87
	09	20.00	52.50	17.50	10.00	98.30 ± 1.20	-10.50 ± 0.40	97.75 ± 0.70

The results are mean values ± SD derived from three different experimental batches. O is denoted for Oil Phase (Labrafac CC®), S for surfactant (Crempohor RH 40®), CoS for co-surfactant (Transcutol P®) and AQ is denoted for aqueous phase (Distilled Water). The DME formulations contain donepezil – 16.67 mg/mL.

4.1.4 Donepezil mucoadhesive microemulsions (DMME):

Donepezil mucoadhesive microemulsions (DMME) were prepared by addition of mucoadhesive agents such as chitosan or Carbopol 934 P (Behl et al. 1998; Ugwoke et al. 2001; Sinsawat et al. 2003; Alpar et al. 2005) to optically clear DME. DME were prepared as described under section 4.1.3 and chitosan was added in a concentration of 0.25% w/w, 0.50% w/w and 1.0% w/w with continuous stirring. The resultant DMME was stirred for 30 min and allowed to hydrate for 12 h. Similarly another set of DMME were prepared by adding Carbopol 934 P to DME in a concentration of 0.25% w/w, 0.50% w/w and 1.0% w/w with continuous stirring. The resultant DMME was stirred for 30 min and allowed to hydrate for 12 h.

4.2 Characterization

4.2.1 Appearance:

Appearances of TME and DME were performed against white and black background. The test was carried out as described in the Indian Pharmacopoeia (1996) and United States Pharmacopoeia (2003).

4.2.2 pH Determination:

The pH of TME and DME was measured by diluting the 10 mL of respective test sample of ME with 10 mL of purified water. The resultant solution/dispersion is stirred for 5 min. The pH is recorded using calibrated digital pH meter at 25° C ± 1°C. The pH was recorded in triplicate when the pH gets stabilized. pH meter was calibrated daily using standard buffer tablet prior to record the observations.

4.2.3 Globule size Determination:

The globule size determination (Roland et al. 2003; Kaler and Prager 1982) of TME and DME were determined using photon correlation spectroscopy (PCS) method with in-built Zetasizer (Model: Nano ZS, Malvern Instruments, UK) at 633 nm. The equipment was equipped with 18 mm width, helium-neon gas laser source having intensity of 4 mW. The mean PCS diameter is the so-called intensity-weighted "z-average" (mean particle size). Average of ten measurements of each sample was used for derivation of mean particle size. Latex dispersion having mean particle size 60 nm ± 5 nm was used as a standard. The standard was evaluated after every 60 min during measurement of test samples in order to validate the equipment.

4.2.4 Zeta Potential Determination:

The Nano ZS zeta seizer was used to measure the zeta potential by electrophoresis and electrical conductivity of the formed ME (Roland et al. 2003) was also performed using in built conductivity option (Zeta potential) of the Zetasizer. The electrophoretic mobility (μm/s) was converted to zeta potential by in-built software using Helmholtz-Smoluchowski equation. Measurements were performed using small volume disposable zeta cell. Average of twenty measurement of each sample was used to derive average zeta

potential. Latex dispersion having zeta potential -50 mV ± 2.5 mV was used as a standard. The standard was evaluated after every 60 min during measurement of test samples in order to validate the results of test formulation.

4.2.5 Active Ingredient Analysis:

TME and DME were analyzed for presence of respective active ingredient using respective method of analysis for analyzing formulations described in chapter 3, sections 3.2 and 3.4.

4.3 Physical Stability

TME and DME were subjected to physical and chemical stability (Kantaria et al. 1999). The prepared ME have been subjected to accelerated centrifugation for the assessment of physical phase separation, if any between the oil and aqueous phase. Some of the batches meeting the criteria mentioned below have been selected (Block et al. 2001)

Criteria for selection of batches

1. Microemulsions having mean globule size below 50 nm; and
2. Zeta potential at least -25 mV

Microemulsions having least globule size are expected to have larger surface area and therefore, may get absorbed or may transverse rapidly across the nasal mucosa. Moreover, literature citation revealed that ME which are negatively charged and having zeta potential close to -25 mV or less exhibits moderate to excellent physical stability (Nash et al. 2001, Roland et al. 2003). Therefore, both the selection criteria were used as a filter prior to assessment of accelerated physical stability. These experimental batches are marked in bold face fonts (within Table 4.2 to 4.5 and Table 4.7 to 4.9).

Method

Approximately 5 mL of the ME was charged to the centrifugation tube and the top of the tube was tightly closed using screw-on cap. Phase separation study of the globule size- and zeta potential-fractionated ME were performed using accelerated centrifugation at 3.6 x 10^6 x g at 4° C ± 2° C temperature for 15 min (Roland et al. 2003). Sample from top, middle and bottom were collected using 24" needle fitted on 1 mL syringe and globule size determination was performed using photon correlation spectroscopy (as mentioned under characterization in this chapter, section 4.2). The results of globule size following accelerated centrifugation for selected batches of TME and DME are recorded in Table 4.10 and 4.11. A few representative batches wherein the physical separation was noticed (different globule size compare to initial values) were also analyzed for active ingredient content to reconfirm the separation. The results of the promising batches with acceptable globule size difference (± 5 nm) as compared to initial values (marked in bold face font in Table 4.10 and 4.11) have been evaluated for chemical stability followed by *in vitro* drug diffusion studies (Khoshnevis et al. 1997; Koziara et al. 2003) using sheep nasal mucosa to assess diffusion coefficient, mean flux rate and release kinetics (Gavini et al. 2005).

Table 4.10 Accelerated physical stability of tacrine microemulsions

System	Ratio of S:CoS	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm)		
							Top layer	Middle layer	Bottom layer
TME 1	3:1	05	15.00	41.25	13.75	30.00	24.39 ± 0.65	22.12 ± 0.37	21.35 ± 0.67
TME 2	3:1	05	15.00	41.25	13.75	30.00	26.76 ± 0.52	25.34 ± 0.35	22.67 ± 0.92
TME 3	3:1	05	15.00	48.75	16.25	20.00	48.74 ± 1.45	46.26 ± 1.24	45.50 ± 1.68
TME 4	3:1	05	15.00	48.75	16.25	20.00	47.53 ± 1.87	44.23 ± 1.22	43.45 ± 0.96

Table 4.11 Accelerated physical stability of donepezil microemulsions

System	Ratio of S:CoS	Formulation	O (%)	S (%)	CoS (%)	AQ (%)	Globule size (nm)		
							Top layer	Middle layer	Bottom layer
DME 1	1:1	04	25.00	20.00	20.00	35.00	81.04 ± 3.23	79.23 ± 3.52	71.56 ± 2.89
DME 1	**1:1**	**05**	**25.00**	**22.50**	**22.50**	**30.00**	**44.05 ± 1.62**	**43.34 ± 1.47**	**40.42 ± 1.13**
DME 2	**3:1**	**05**	**15.00**	**41.25**	**13.75**	**30.00**	**25.35 ± 0.87**	**22.87 ± 0.72**	**22.05 ± 0.64**
DME 3	**3:1**	**05**	**15.00**	**48.75**	**16.25**	**20.00**	**48.17 ± 1.43**	**47.52 ± 1.28**	**43.67 ± 1.15**

4.4 Chemical Stability (Drug Retention Studies)

Tacrine and donepezil microemulsions were subjected to accelerated temperature and stress conditions (**http://www.nihs.go.jp/dig/ich/quality/q1e/Q1E**). The ME were analyzed for physical and chemical stability. Approximately 10 mL of the formulation was filled in USP type III glass vials and sealed using VP6 crimp on spray pump fitted with 10 µm actuator. Physical stability was assessed using accelerated centrifugation technique as described previously in this chapter (section 4.4, Roland et al. 2003).

The stress stability was conducted at 60° C ± 2° C in an incubator. The accelerated stability was performed at 30° C ± 2° C / 65% ± 5% relative humidity (RH) and 40° C ± 2° C / 75% ± 5% RH. The duration of stability was 6 months and samples were withdrawn at predetermined time intervals after 1 month, 2 months, 3 months and 6 months (**http://www.nihs.go.jp/dig/ich/quality/q1e/Q1E**). The parameters such as physical separation at accelerated gravitational force, active ingredient content, globule size determination, zeta potential measurement, appearance, cracking or physical separation, solidification/ gel formation etc. were assessed. These parameters were evaluated as per the methods described in the section 4.2 and 4.3. The results for tacrine and donepezil microemulsions drug retention studies are recorded in Table 4.12, and 4.13.

Table 4.12 Accelerated chemical stability of tacrine microemulsions at 40°C/75% RH and 30°C/65% RH

System	Ratio of S:CoS	O (%)	S (%)	CoS (%)	AQ (%)	Period (month)	40°C/75% RH				30°C/65% RH			
							Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD
TME 1	3:1	15.00	41.25	13.75	30.00	0	23.50 ± 0.40	-20.50 ± 0.51	99.78 ± 0.42	99.67 ± 0.35	23.50 ± 0.40	-20.50 ± 0.51	99.78 ± 0.42	99.67 ± 0.35
						1	24.52 ± 0.33	-20.87 ± 0.43	99.63 ± 0.35	99.18 ± 0.44	22.85 ± 0.44	-21.02 ± 0.37	99.82 ± 0.26	99.45 ± 0.29
						2	22.42 ± 0.39	-21.39 ± 0.69	99.70 ± 0.40	98.87 ± 0.50	24.15 ± 0.32	-20.84 ± 0.40	99.62 ± 0.34	98.82 ± 0.63
						3	23.13 ± 0.56	-21.85 ± 0.34	99.61 ± 0.51	98.12 ± 0.61	24.65 ± 0.48	-21.50 ± 0.58	99.69 ± 0.39	98.36 ± 0.40
						6	24.76 ± 0.65	-22.88 ± 0.46	99.84 ± 0.48	97.56 ± 0.86	25.58 ± 0.42	-22.66 ± 0.62	99.76 ± 0.53	97.25 ± 0.44
TME 2	3:1	15.00	41.25	13.75	30.00	0	24.00 ± 0.87	-19.37 ± 1.27	99.12 ± 0.25	99.35 ± 0.32	24.00 ± 0.87	-19.37 ± 1.27	99.12 ± 0.25	99.35 ± 0.32
						1	24.45 ± 0.63	-21.12 ± 1.18	99.46 ± 0.64	99.05 ± 0.46	23.58 ± 0.52	-18.18 ± 1.07	99.34 ± 0.43	99.45 ± 0.85
						2	25.64 ± 0.86	-19.67 ± 1.28	99.30 ± 0.24	98.77 ± 0.45	22.89 ± 0.84	-20.49 ± 0.96	99.64 ± 0.29	99.02 ± 0.64
						3	23.82 ± 0.77	-20.53 ± 1.36	99.05 ± 0.35	98.38 ± 0.34	25.80 ± 0.60	-21.27 ± 1.24	99.41 ± 0.51	98.36 ± 0.77
						6	26.14 ± 0.54	-22.43 ± 0.94	99.34 ± 0.28	97.62 ± 0.57	26.45 ± 0.44	-22.92 ± 1.62	99.16 ± 0.45	97.16 ± 0.87

Table 4.12 Accelerated chemical stability of tacrine microemulsions at 40°C/75% RH and 30°C/65% RH (Contd...)

System	Ratio of S:CoS	O (%)	S (%)	CoS (%)	AQ (%)	Period (month)	40°C/75% RH				30°C/65% RH			
							Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD
TME 3	3:1	15.00	48.75	16.25	20.00	0	44.00 ± 0.95	-18.95 ± 0.68	99.25 ± 0.40	99.81 ± 0.56	44.00 ± 0.95	-18.95 ± 0.68	99.25 ± 0.40	99.81 ± 0.56
						1	43.45 ± 0.66	-20.36 ± 0.43	99.48 ± 0.34	99.22 ± 0.44	46.12 ± 1.41	-19.34 ± 0.45	99.62 ± 0.55	99.56 ± 0.85
						2	45.70 ± 1.12	-20.17 ± 0.29	99.36 ± 0.42	98.67 ± 0.73	44.56 ± 0.87	-18.23 ± 0.64	99.33 ± 0.42	99.11 ± 0.46
						3	46.12 ± 0.87	-19.62 ± 0.35	99.17 ± 0.56	99.05 ± 0.49	45.47 ± 1.13	-20.14 ± 0.75	99.46 ± 0.39	98.65 ± 0.90
						6	48.26 ± 1.27	-21.04 ± 0.62	99.25 ± 0.31	97.78 ± 0.83	47.68 ± 1.52	-22.68 ± 0.47	99.14 ± 0.23	97.11 ± 1.14
TME 4	3:1	15.00	48.75	16.25	20.00	0	42.36 ± 1.27	-19.62 ± 0.85	99.12 ± 0.25	99.67 ± 0.59	42.36 ± 1.27	-19.62 ± 0.85	99.12 ± 0.25	99.67 ± 0.59
						1	40.94 ± 1.45	-19.02 ± 0.32	99.25 ± 0.42	98.87 ± 0.79	43.34 ± 1.14	-20.43 ± 0.56	99.43 ± 0.36	99.23 ± 0.34
						2	43.71 ± 1.54	-20.69 ± 0.54	99.06 ± 0.38	99.32 ± 1.09	44.25 ± 0.97	-19.43 ± 0.65	99.35 ± 0.22	99.56 ± 0.63
						3	42.92 ± 1.12	-20.04 ± 0.38	99.28 ± 0.40	98.24 ± 0.88	43.82 ± 1.12	-20.12 ± 0.41	99.50 ± 0.47	98.23 ± 0.68
						6	45.16 ± 1.22	-22.92 ± 0.63	99.27 ± 0.16	97.46 ± 1.25	45.87 ± 1.47	-21.75 ± 0.76	99.24 ± 0.33	97.06 ± 0.94

Table 4.13 Accelerated chemical stability of donepezil microemulsions at 40°C/75% RH and 30°C/65% RH

System	Ratio of S:CoS	O (%)	S (%)	CoS (%)	AQ (%)	Period (month)	40°C/75% RH				30°C/65% RH			
							Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD
DME 1	1:1	25.00	22.50	22.50	30.00	0	41.58 ± 1.51	-23.49 ± 1.48	99.44 ± 0.29	99.54 ± 0.82	41.58 ± 1.51	-23.49 ± 1.48	99.44 ± 0.29	99.54 ± 0.82
						1	40.88 ± 1.12	-25.12 ± 0.83	99.30 ± 0.24	98.78 ± 1.05	42.20 ± 1.28	-23.20 ± 1.23	99.62 ± 0.34	99.76 ± 0.64
						2	42.06 ± 1.32	-24.34 ± 0.96	99.55± 0.35	99.12 ± 0.66	42.65 ± 0.87	-22.15 ± 0.74	99.33 ± 0.38	99.35 ± 0.44
						3	43.44 ± 1.67	-25.33 ± 1.16	99.46 ± 0.52	98.38 ± 1.24	43.23 ± 1.19	-24.87 ± 1.13	99.47 ± 0.45	99.12± 1.04
						6	45.23 ± 1.45	-27.17 ± 1.29	99.26 ± 0.32	97.78 ± 1.31	44.71 ± 1.43	-26.67 ± 1.42	99.16 ± 0.41	98.04 ± 1.34
DME 2	3:1	15.00	41.25	13.75	30.00	0	23.50 ± 0.40	-20.50 ± 0.51	99.78 ± 0.42	99.34 ± 1.31	23.50 ± 0.40	-20.50 ± 0.51	99.78 ± 0.42	99.34 ± 1.31
						1	22.78 ± 0.68	-19.45 ± 0.40	99.84 ± 0.64	98.83 ± 0.91	24.33 ± 0.75	-19.86 ± 0.41	99.57 ± 0.34	99.45 ± 1.18
						2	23.88 ± 0.59	-20.21 ± 0.38	99.56 ± 0.51	99.06 ± 1.40	24.54 ± 0.68	-20.77 ± 0.35	99.52 ± 0.30	98.87 ± 1.43
						3	24.59 ± 0.64	-20.87 ± 0.45	99.22 ± 0.40	98.03 ± 1.22	25.10 ± 0.55	-21.56 ± 0.73	99.34 ± 0.44	98.12 ± 1.50
						6	26.53 ± 1.08	-22.43 ± 0.34	99.42 ± 0.39	97.24 ± 1.46	25.88 ± 0.40	-23.34 ± 0.56	99.42 ± 0.23	97.11 ± 1.27

Table 4.13 Accelerated chemical stability of donepezil microemulsions at 40°C/75% RH and 30°C/65% RH (Contd...)

System	Ratio of S:CoS	O (%)	S (%)	CoS (%)	AQ (%)	Period (month)	40°C/75% RH				30°C/65% RH			
							Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD	Globule size (nm) ± SD	Zeta potential (mV) ± SD	Trans-mittance (%) ± SD	Drug content (%) ± SD
DME 3	3:1	15.00	48.75	16.25	20.00	0	44.00 ± 0.95	-18.95 ± 0.68	99.25 ± 0.40	99.12 ± 1.03	44.00 ± 0.95	-18.95 ± 0.68	99.25 ± 0.40	99.12 ± 1.03
						1	44.56 ± 0.86	-18.67 ± 0.45	99.40 ± 0.25	98.77 ± 0.88	43.74 ± 0.68	-19.23 ± 0.55	99.33 ± 0.22	99.35 ± 0.85
						2	43.88 ± 0.75	-19.23 ± 0.36	99.34 ± 0.31	98.10 ± 0.67	44.87 ± 0.56	-19.68 ± 0.77	99.50 ± 0.35	98.90 ± 0.92
						3	45.12 ± 0.82	-20.45 ± 0.56	99.26 ± 0.20	97.45 ± 1.20	45.10 ± 0.83	-20.12 ± 0.60	99.28 ± 0.40	98.14 ± 0.83
						6	47.40 ± 0.68	-22.12 ± 0.78	99.45 ± 0.35	96.67 ± 1.13	46.60 ± 0.70	-21.40 ± 0.75	99.40 ± 0.29	97.25 ± 1.12

4.5 Results and Discussion

Tacrine microemulsions were successfully prepared using the titration technique followed by construction of pseudo-ternary phase diagrams (Lianli et al. 2002). To screen out a drug vehicle suitable for intranasal delivery of tacrine, four different ME systems were prepared wherein system 1 and 2 comprise of Labrafil M 1944 CS® as an oil phase, Transcutol P® as a co-surfactant and distilled water as an aqueous phase. Cremophor RH 40® and Cremophor EL® were used as surfactant for system 1 and system 2 respectively. Similarly, system 3 and system 4 were formulated in the identical manner as system 1 and system 2 respectively by replacing Labrafil M 1944 CS® with Labrafac CC®. Microemulsion formation was spontaneous upon addition of aqueous phase to drug in oil-surfactant-co-surfactant mixture. The solubility data shown that tacrine has maximum solubility in Labrafil M 1944 CS® (> 35 mg/mL) and Labrafac CC® (> 10 mg/mL) therefore; these oils are selected to formulate ME. However, with castor oil, corn oil, sunflower oil and isopropyl myristate, the solubility of drug in oil was less than 10 mg/mL. Moreover, nasal formulations are concentrated preparations as low volumes can be administered into the nostril (< 200 µL), the ME base was selected on the merits of solubilization capacity of tacrine. The selection of surfactant and co-surfactant mixture was on the basis of HLB values. The mixtures reported in literature and which can provide HLB value between 9 and 12 were selected. Phase studies were done to investigate the effect of S:CoS ratio on the existence ranges of stable o/w ME region. Microemulsions were formulated at different S:CoS ratio such as 1:1, 2:1 and 3:1. The pseudo-ternary phase diagrams with various S:CoS weight ratios for TME 1, TME 2, TME 3, and TME 4 are displayed in Figs. 4.2, 4.3, 4.4, and 4.5 respectively. The transparent ME area is presented in the phase diagrams as shaded region. No distinct conversion from w/o to o/w ME was seen; therefore, this single isotropic region is considered as a bicontinuous ME. The rest of the region on the phase diagram represents the viscous gel area or turbid and conventional emulsions based on visual identification. From these phase diagrams, the influence of relative S:CoS concentrations on the ME isotropic region can be evidently seen. The phase study revealed that with all four systems, changing S:CoS ratio from 1:1 to 3:1, the ME region increased in size with the higher surfactant concentration. This increase was toward the oil–water axis, indicating that by increasing the surfactant concentration, the maximum amount of water and oil that could be solubilized into the ME increased. These results are consistent with those

reported earlier by Lianli et al. (2002) and Zhang et al. (2004). From a formulation viewpoint, the increased oil content in ME may provide a greater opportunity for the solubilization of drug. The viscosities of ME were also affected by the surfactant content. With the higher weight percentage of surfactant, the viscosities of the ME formulation increased, and a gel formation was observed. For nasal delivery, a less viscous ME is preferred considering the requirement of sprayability of nasal formulation by the pump device and the dispersion uniformity of the spray.

The globule size and zeta potential data for selected compositions of TME (System 1 to 4) are recorded in Table 4.2, 4.3, 4.4 and 4.5. At large, it was observed from the data that increase in concentration of oil phase, resulted in increase in globule size. This may be due to fact that part of the oil phase may not form micelles. The globule size and zeta potential were fairly reproducible within ± 5 nm / ± 2 mV range respectively. Comparing globule size of different formulations with varying concentrations of S:CoS mixture, it was observed that increase in the S:CoS mixture concentrations results in the increase in the globule size. Therefore, it was concluded that the concentration of S:CoS mixture may be critical for the formation of TME (Nash et al. 2001). Increase in the concentration and the ratio of surfactant to co-surfactant, resulted into formation of bicontinuous ME or o/w ME. It was also observed that increase in the aqueous phase concentration resulted in decrease in the zeta potential (anionic). Reports in the literature revealed that ME having zeta potential more than 25 mV absolute value exhibit moderate to best physical stability in terms of phase separation (Rôland et al. 2003). Therefore, ME having zeta potential close to -25 mV or less were selected for further studies (Nash et al. 2001; Block et al. 2001). Also, ME with less globule size may have larger surface area and better permeation across the mucosal interstitial spaces. Therefore, globule size of 50 nm was identified as a filter for the selection criteria for further studies. Comparing TME 1 and TME 2 or TME 3 and TME 4 for the ME region (Figure 4.2 and 4.3 respectively or Figure 4.4 and 4.5 respectively), the ME region obtained with Cremophor RH 40® was found to be wider in comparison to those obtained with Cremophor EL®. This may be attributed to the lower HLB value of Cremophor RH 40® which is responsible for more oil solubilization. Comparing TME 1 and TME 2 vs. TME 3 and TME 4, it was observed that the S:CoS systems used produced smaller ME regions when Labrafac CC® was used as an oil phase. The oil concentrations in excess of 30% w/w did not yielded ME like system 1 and system 2. Further, the globule sizes and zeta potentials obtained for system

3 and system 4 were more compared to system 1 and system 2. A few of the batches of TME 3 (Formulations 04, 07, 08,and 09) and TME 4 (Formulations 04, 07 and 08) show globule size greater than 200 nm which were visually hazy in appearance and in addition to that, it showed percent transmittance less than 98%. It was found that with increase in the oil concentration, the globule size also increased significantly and lead to poor transmittance (less than 98%). At large, the ME were found to be clear, transparent (transmittance > 99% at 630 nm), spontaneous formation and either of o/w or bicontinuous for all four systems of tacrine microemulsions. The prepared ME of tacrine having globule size less than 50 nm and zeta potential close to -25 mV or less have been further evaluated for physical stability and chemical stability.

Donepezil microemulsions were successfully prepared using titration technique and the ME regions are plotted using pseudo-ternary phase diagrams (Lianli et al. 2002). To screen out a drug vehicle suitable for intranasal delivery of donepezil, three different DME systems (DME 1, DME 2, and DME 3) were prepared wherein system 1 comprises of Captex 355® as oil phase, Tween 80 as surfactant, Capmul MCM® as co-surfactant and distilled water as an aqueous phase. System 2 and system 3 were prepared using Labrafil M 1944 CS® and Labrafac CC® an oil phase respectively, Cremophor RH 40® as surfactant, Transcutol P® as a co-surfactant and distilled water as an aqueous phase. The oils were selected on the merit of highest solubility of donepezil which is performed using biopharmaceutical classification system solubility studies. Microemulsion formation was found to be spontaneous upon addition of aqueous phase to drug in oil-surfactant-co-surfactant mixture and the prepared ME were visually clear and transparent. The percent transmittances were measured using spectrophotometer (Shimadzu UV-1601, Japan) at 630 nm and the results are recorded in Table 4.7, 4.8, and 4.9. As seen from the results, all DME systems showed transmittance > 99% for all batches of ME prepared except for few batches of DME 3. The SD data for transmittance for six different batches indicated that process was reproducible and all the values are within the range of ±1 %. Microemulsions for all the systems were prepared at S:CoS ratios 1, 2 and 3. The globule size and zeta potential are recorded in Table 4.7, 4.8, and 4.9. The globule sizes were found within ± 5 nm of the estimated globule size which indicates uniform globule size distribution and narrow distribution for all the batches. Zeta potentials were also fairly reproducible within ± 2 mV range. Increase in the oil concentration to highest resulted in increase in cationic charge and the system shows net positive charge compared to other

batches. However, apparently no size separation was noticed immediately after formation of ME. This may be attributed to the fact that increase in the oil concentration may lead to reduction in the concentration of aqueous phase (water) which may result in poor conductivity and increased zeta potential (Espinoza-Jimenez et al. 2003). Moreover, it was also found that increase in the total concentration of S:CoS (up to medium range), absolute zeta potential increases. This may be due to increase in the concentration of co-surfactant result into formation of bicontinuous or o/w system due to higher HLB value hence, the negative charge of the system also increases (Nash et al. 2002). At higher concentrations of S:CoS absolute zeta potential decreases due to substantial decrease in aqueous phase. Comparing DME 2 and DME 3, it was observed that the S:CoS systems used produced smaller ME regions when Labrafac CC® was used as an oil phase. The oil concentrations in excess of 30% w/w did not yielded ME like DME 2. Further, the globule sizes and zeta potentials obtained for DME 3 were more compared to DME 2. A few of the batches of DME 3 show globule size greater than 200 nm which were visually hazy in appearance and in addition to that, it showed percent transmittance less than 98% (System 3, Formulation 04, 07, 08 and 09). It was found that with increase in the oil concentration, the globule size also increased significantly and lead to poor transmittance (less than 98%). At large donepezil microemulsions were found to be clear, transparent (transmittance > 99% at 630 nm), spontaneous formation and either of o/w or bicontinuous for all three systems. The prepared ME of donepezil having globule size less than 50 nm and zeta potential close to -25 mV have been further evaluated for physical stability and chemical stability.

The ME of both the drug substances were prepared successfully and the phase regions delineating phase boudaries were succeessfully plotted in a psedo-ternary phase diagrams. Microemulsions of tacrine and donepezil were subjected to accelerated centrifugation for assessing the stability of the formed microemulsions. As seen from Table 4.10, four different batches of TME were subjected for the assessment of physical stability. The data revealed that there was no appreciable change before and after centrifugation for 15 min at accelerated conditions. Moreover, the layers from top, middle and bottom following centrifugation were sampled and analyzed to determine homogeneity. The globule size of the TME in top, middle and bottom layer for different formulations were within ± 5 nm from the initial values. The data clearly suggested that tacrine microemulsions were found physically stable under the testing conditions. The ME were selected on the basis of

globule size. All the batches of ME were having globule size less than 50 nm and zeta potential close to -25 mV or less. It was also observed that ME having zeta potential close to or less than -25 mV gives reasonably good physical stability with regards to phase separation. For donepezil microemulsions, four different batches having particle size less than 50 nm and zeta potential close to -25 mV were selected for the studies. The data before and after centrifugation were recorded in Table 4.11. As seen from the data, significant increase and difference in the globule size and size distribution of a DME 1, Formulation 04 was observed. It was also observed that the total concentration of surfactant and co-surfactant mixture was less in DME 1, formulation 04 as compared to DME 1, formulation 05. Thus, lower concentration of S:CoS mixture may result into spontaneous formation of ME however, it is indicative of phase separation on aging. The top layer showed higher globule size compared to middle and bottom layer, this may be due to separation of oil and floating on the top layer due to low bulk density compare to aqueous phase. Moreover, globule sizes in the bottom layer was found similar to the initial values, this is indicative that the part quantity of oil phase gets separated and remaining oil phase gets emulsified by the surfactant: co-surfactant used in the formulation. It was concluded that physical stability assessment can be successfully performed using accelerated centrifugation technique by sampling the ME from top, middle and bottom layers. Microemulsions which are bicontinuous, w/o or o/w were found to be stable.

Drug retention study were performed on physically stable ME by subjecting tacrine and donepezil microemulsions at 30°C / 65% RH and 40°C / 75% RH. The data were recorded in Table 4.12 and 4.13 for tacrine and donepezil microemulsions respectively. As seen from the table 4.12, tacrine microemulsions were assessed for globule size, size distribution, zeta potential, percent transmittance, and drug content. When globule size was evaluated up to six months, it was found that globule size for all four TME formulations were within the range of ± 5 nm from the initial values and no abnormal changes in the globule size were noticed at both the accelerated testing conditions. The zeta potential values were also found to be consistent and within the range ± 5 mV from the initial values. The data clearly indicated that the formulations were physically stable at 30°C / 65% RH and 40°C / 75% RH without noticeable change in the zeta potential values. Percent transmittances for all the experimental batches were found to be greater

than 99% which indicated the clarity of the tested ME and indirectly gives an indication that no separation was observed in the tacrine microemulsions. Drug content for different TME formulations were found to be more than 95 % of the label claim (33 mg/mL). The data clearly demonstrated that there was no appreciable degradation at 30°C / 65% RH and 40° C/ 75% RH. The results of tacrine microemulsions demonstrated that the formulations are physically and chemically stable at accelerated stability conditions. The formulations were found to meet the general monograph of Pharmacopoeia and criteria stipulated therein for the liquid preparations. Drug retention studies for donepezil microemulsions were also performed as mentioned under ICH-Q1E guidance. The stability data are recorded in Table 4.13. As seen from the data, the globule size and size distribution remain unchanged after 6 months at both the testing conditions for all the formulations of donepezil. The globule size for DME 1, DME 2, and DME 3 were found within the range of ± 5 nm from the initial values. No appreciable change was noticed when compared with the initial values. Zeta potential also were found to be consistent with the initial observations and the values were within ± 5 mV compared to the initial values indicated the physical stability of the oil/S:CoS/aqueous interface of the prepared ME. The globule size data and zeta potential values clearly pointed-out that the prepared ME of donepezil were physically stable at both the accelerated conditions and the systems were found to be thermodynamically stable. The percent transmittance at 630 nm was found to be greater than 99% indicated the clarity of the emulsions. Values greater than 99% also suggest that there was no inversion, phase separation or cracking of the prepared ME of donepezil. It is evident from the dug retention data that degradation was well within the criteria stipulated by the Indian Pharmacopoeia. The content of the donepezil was found to be within ± 5% of the labeled claim (16.67 mg/mL).

Studies of this investigation conclusively demonstrated that ME were successfully prepared using different oil, S:CoS systems for tacrine and donepezil. The titration method was used for delineating the phase boundaries and pseudo-ternary phase diagrams were constructed to precisely and comprehensively study the phase behavior of the prepared ME systems. Formulations were characterized using photon correlation spectroscopy for globule size, size distribution and zeta potential. The clarity was also evaluated in order to confirm the formation of ME. The globule size data suggested that ME were formed and the globule sizes were found to be less than 200 nm (Marttin et al.

1997). Microemulsions having zeta potential close to or less than -25 mV exhibited good physical stability. Furthermore, the prepared ME were subjected to accelerated centrifugation to accelerate phase separation. The physical separation technique was successfully used as a filter for the selection of batches for further evaluation. Consequently, the promising batches were subjected to accelerated stability studies as per ICH guidelines and formulations were evaluated for all the parameters at different time intervals. Physically and chemically stable ME were further taken up for the *in vitro* diffusion studies to evaluate the potential. Moreover, the ME were admixed with the mucoadhesive agents and comparative *in vitro* diffusion including the diffusion kinetics was evaluated (Willimann et al. 1992).

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