Chapter 4

Preparation of microemulsions

4.1. Introduction

Microemulsion systems, owing to their pharmaceutical advantages (thermodynamic stability, ease of preparation, transparency, low viscosity, considerable potential for solubilization of variety of drugs) are the object of investigations in relation to drug delivery. In spite of numerous advantages in comparison with other colloidal vehicles, microemulsions often require a high content of surfactant. The concentration of surfactant can sometimes be reduced by the addition of cosurfactants. In order to formulate microemulsion drug delivery system solubility of the drug in the different solvents was determined.

4.2. Experimental:

4.2.1. Drugs

Acyclovir was kindly gifted by Alembic Ltd, Vadodara. Efavirenz was kindly gifted by Ranbaxy Ltd, Dewas, India

4.2.2. Reagents:

Labrasol (Caprylocapryl macrogol-8-glyseride), Plurol Olique (Polyglycerol 6-dioleate), Labrafac (Medium chain triglycerides), Labrafil M 1944 CS (oleeoyl macrogol-6-glycerides EP), labrafac Hydro (mixture of mono-, di- and triglycerides and mono- and di- fatty acid esters of polyethylene glycol), Labrafac Lipo (Medium chain triglycerides EP), transcutol (diethylene glycol monoethylether) Glyceryl palmitostearate (Precirol ATO 5), Lauroglycol FCC (propylene glycol laurate), miglyol 812 was received from Colorcon Asia Pvt. Ltd. (mfg: Gattefosse, France) , Cremophor RH 40 (polyethoxylated hydrogenated castor oil), Polaxomer 188 (poly(oxyethylene), poly(oxypropylene) block polymers) was purchased from BASF corporation, Tween 80 (Polyoxyethylene sorbitan monooleate), Propylene glycol, PEG 400, Sodium Lauryl sulphate (SLS), Triacetin, Oleic acid was purchased from SD fine chemicals, India. Sunflower oil, Stearic acid, Glyceryl mono-stearate, Glyceryl tristearate was purchased from National chemical Ltd, India.

4.2.3. Equipments:

Remi magnetic stirrer 1MLH (Remi Equipments, Mumbai, India), Shimadhu UV-1601 UV-Visible spectrophotometer (Shimadju corporation, Kyoko, Japan), Vortex mixer (Remi Equipments, Mumbai, India), spinning drop interfacial tensiometer (Model 500, assembled at The University of Texas).

4.3. Solubility of acyclovir and efavirenz in different oil phase and in surfactant:

An excess quantity of drug was added in a separate test tube containing 2 ml of different oil phase, viz labrafac cc, labrafac lipo, labrafac hydro, labrafil M 1944 CS, labrafil M2125 CS, miglyol 812, benzyl alcohol, sun flower oil, soybean oil, lauroglycol FCC. Similarly, an excess quantity of drug was added in a separate test tube containing 10% (w/w) surfactant solution of Tween 80, Labrasol, cremophor RH40, propylene glycol, PEG400, transcutol, SLS, Polaxomer 188, triacetin etc.

The resultant mixer was thoroughly mixed by vortex mixer and then resultant mixer was kept in auto shaker for continuous shaking. Mixing was continued for 72 hours. Then the mixer was centrifuged at 10,000rpm for 20min using Remi cooling centrifuge to separate the un-dissolved drug. The supernatant solution was taken for analysis of the drug. The concentration of drug in the different oil phase was determined using UV-Visible spectrophotometer as described earlier.(Table 2). Briefly the oil or surfactant phase containing drug was dissolved in ethanol or methanol for acyclovir and efavirenz respectively, and the absorbance of the drug was found by keeping the respective blank and solubility was calculated.

4.4. Interfacial tension measurements:

After finding the suitable oil and surfactant, the appropriate surfactant (ST) and cosurfactant (COST) and their ratio was found out by estimating the interfacial tension and by phase diagram study. The microemulsion system under investigation was subjected to interfacial tension measurements using the spinning drop interfacial tensiometer. The measurements were performed by injecting the oil/surfactant or oil/cosurfactant or oil/ ST + COST mixture into the tensiometer capillary filled with water and maintained at a temperature of 30 ± 0.5^0C by oil circulation. Measurements of

the drop diameter were achieved at different speeds ranging from 1000 to 3500 rpm. Density of the different oil phase was measured using a method based on the Archimedean principle as reported in the product literature.

4.5. Preparation of microemulsion:

At first ratio of surfactant to cosurfactant was kept constant. Then required quantity of oil phase was taken in screw-capped test tube. Then known quantity of drug was mixed and dissolved into it by vortexing. Then required quantity of surfactant and cosurfactant at a fixed ratio was added into the above mixture, which was followed by through mixing. The resulted mixture was titrated against distilled water to check the transparency of the system. The mixture was shaken after each addition of water for a short time (about 1 min) by hand or by using a Vortex mixer. The experiment was carried out at room temperature (25 ± 2 °C). Since only the single-phase microemulsion region was of important no attempt was made to obtain the other phases. Blank microemulsion system also prepared using same method.

Table 4.1: Different microemulsion system for detailed studies.

	Acyclovir		Efavirenz	
	System A	System B	System C	System D
Surfactant:	Labrasol	Tween 80,	Labrasol	Cremophor RH 40
Cosurfactant:	Plurol olique	Propylene glycol,	Transcutol	Propylene glycol,
Oil	labrafac	Labrafac	Labrafil M 1944CS	Labrafil M 1944C
Aqueous phase:	D. water	D. water	D. water	D. water

4.6. Construction of phase diagram:

Pseudoternary phase diagram were constructed keeping the ratio of surfactant and cosurfactant constant and varying the remaining two components. As a convenient method, the construction of the phase diagrams were done by drawing 'water dilution lines' representing an increase of water content while decreasing surfactant-cosurfactant

levels (N. Gatri, 2000). The water was titrated along dilution lines drawn from the surfactant-cosurfactant apex (100% surfactant-cosurfactant) to the opposite oil side of the triangle. The line was arbitrarily denoted as the value of the line intersection with the oil scale (e.g. 20:80, 30:70, etc.). In case turbidity appeared followed by a phase separation, the samples were considered as biphasic. In case monophasic, clear and transparent mixtures were visualized after stirring; the samples were marked as points in the phase diagram. The area covered by these points was considered as the microemulsion region of existence.

4.6.1. Acyclovir:

Different surfactant to cosurfactant ratio (km) like 4:1, 3:1, 2:1, 1:1, 1:2, 1:0 (no surfactant) was fixed and corresponding mixture was made by vortexing. This process could identify the transparency zone of the system. Pseudo-ternary phase diagrams were constructed for the different ratio of surfactant to cosurfactant.

4.6.1.1. Phase diagram for labrasol, plurol olique, labrafac and water system (System A):

To determine the microemulsion area phase diagram was constructed using labrasol (HLB=14) as surfactant. Plurol Olique (HLB= 6), labrafac was used as cosurfactant and oil respectively. The following batches (Table 4.2) were made and checked for transparency.

Table 4.2: Formulation variables to check the transparency of the system.(system A)

Batch no	Ratio of ST to COST	% Of[1]		Transparency ranges on dilution with water*	
		STmix	Oil	From	Upto
A-01	4:1	90	10	14	150
A-02	4:1	80	20	11	106
A-03	4:1	70	30	10	38
A-04	4:1	60	40	8	17
A-05	4:1	50	50	4	6
A-06	4:1	40	>60	No transparency observed	
A-07	3:1	90	10	20	175
A-08	3:1	80	20	17	142
A-09	3:1	70	30	14	44
A-10	3:1	60	40	12	28
A-11	3:1	50	50	9	20
A-12	3:1	40	>60	No transparency observed	
A-13	2:1	90	10	30	285
A-14	2:1	80	20	24	230
A-15	2:1	70	30	20	62
A-16	2:1	60	40	14	35
A-17	2:1	50	50	9	17
A-18	2:1	40	60	3	11
A-19	2:1	30	>70	No transparency observed	
A-20	1:1	90	>10	No transparency observed	
A-21	1:2	90	>10	No transparency observed	
A-22	1:0	90	>10	No transparency observed	

[1] Mixture of surfactant (ST) and cosurfactant represented as STmix. % Of STmix and oil was calculated with respect to the total quantity of STmix and oil.

* Calculation of water composition was based on the total quantity of STmix and oil mixture and represented as parts.

4.6.1.2. Phase diagram for Tween 80, propylene glycol, labrafac and water system (System B):

To determine the microemulsion area phase diagram was constructed. Tween 80 (HLB=15) was used as surfactant. Propylene glycol was used as cosurfactant. Labrafac was used as oil phase. The following batches were made (Table 4.3) and check the transparency.

Table 4.3: Formulation variables to check the transparency of the system (system B)

Batch no	Ratio of ST to COST	% Of[d]		Transparency ranges on dilution with water*	
		STmix	Oil	From	Upto
B-01	4:1	90	10	Thick mass obtained.	
B-02	3:1	90	10	1	>500
B-03	3:1	80	20	1	>500
B-04	3:1	70	30	1	143
B-05	3:1	60	40	1	82
B-06	3:1	50	50	1	20
B-10	3:1	40	>60	No transparency observed	
B-11	2:1	90	10	1	>500
B-12	2:1	80	20	1	>500
B-13	2:1	70	30	1	80
B-14	2:1	60	40	1	48
B-15	2:1	50	50	1	21
B-19	2:1	40	>60	No transparency observed	
B-20	1:1	90	10	1	> 500
B-21	1:1	80	20	1	160
B-22	1:1	70	30	1	55
B-23	1:1	60	40	1	20
B-28	1:1	50	>50	No transparency observed	
B-29	1:2	90	10	1	>500
B-30	1:2	80	20	1	72
B-31	1:2	70	30	1	20

Cont.....

B-37	1:2	60	>40	No transparency observed
B-38	1:0	90	>10	Thick mass obtained. No transparency

[1] Mixture of surfactant (ST) and cosurfactant represented as STmix. % of STmix and oil was calculated with respect to the total quantity of STmix and oil.

* Calculation of water composition was based on the total quantity of STmix and oil mixture and represented as parts.

4.6.2. Efavirenz:

Different surfactant to cosurfactant ratio (km) like 4:1, 3:1, 2:1, 1:1, 1:2 and 1:0 (no cosurfactant) was fixed and corresponding mixture was made followed by vortexing. This process found out the transparency zone of the system. Pseudo-ternary phase diagrams were constructed for the different ratio of surfactant to cosurfactant.

4.6.2. 1. Labrasol, transcutol, labrafil M 1944 CS and water system (System C)

To determine the microemulsion area phase diagram was constructed using labrasol (HLB= 16) as surfactant. Transcutol, labrafil M 1944 CS was used as cosurfactant and oil respectively. The following batches were made (Table 4.4) and check the transparency.

Table 4.4: Formulation variables to check the transparency of the system (system C)

Batch no	Ratio[1] of ST to COST	% Of[2]		Transparency ranges on dilution with water*	
		STmix	Oil	From	Upto
C-01	4:1	90	10	1	>500
C-02	4:1	80	20	1	>500
C-03	4:1	70	30	1	225
C-04	4:1	60	40	1	159
C-05	4:1	50	50	1	88
C-06	4:1	40	60	1	34
C-07	4:1	30	>70	No transparency	
C-08	3:1	90	10	1	>500
C-09	3:1	80	20	1	>500
C-10	3:1	70	30	1	142

Batch no	Ratio[1] of ST to COST	% Of[2]		Transparency ranges on dilution with water*	
		STmix	Oil	From	Upto
C-11	3:1	60	40	1	96
C-12	3:1	50	50	1	40
C-13	3:1	40	>60	No transparency	
C-14	2:1	90	10	1	>500
C-15	2:1	80	20	1	>500
C-16	2:1	70	30	1	68
C-17	2:1	60	40	1	42
C-18	2:1	50	50	1	18
C-19	2:1	40	>60	No transparency observed	
C-20	1:1	90	10	1	>500
C-21	1:1	80	20	1	122
C-22	1:1	70	30	1	35
C-23	1:1	60	40	1	15
C-24	1:1	50	>50	No transparency observed	
C-25	1:2	90	10	1	>500
C-26	1:2	80	20	1	82
C-27	1:2	70	30	1	12
C-28	1:2	60	>40	No transparency observed	
C-29	1:0	90	>10	Thick mass obtained. No transparency	

[1] Mixture of surfactant (ST) and cosurfactant represented as STmix. % Of STmix and oil was calculated with respect to the total quantity of STmix and oil.

* Calculation of water composition was based on the total quantity of STmix and oil mixture and represented as parts.

4.6.2. 2. Cremophor RH 40, propylene glycol, labrafil M 1944C and water system (System D):

To determine the microemulsion area phase diagram was constructed using cremophor RH 40 (HLB= 16) as surfactant. Propylene glycol and Labrafil M 1944 CS was used as

cosurfactant and oil respectively. The following batches (Table 4.5) were made and check the transparency.

Table 4.5: Formulation variables to check the transparency of the system (system D)

Batch no	Ratio[1] of ST to COST	% Of[2]		Transparency ranges on dilution with water*	
		STmix	Oil	From	Upto
D-01	4:1	90	>10	No transparency. Thick mass obtained	
D-02	3:1	90	10	1	>500
D-03	3:1	80	20	1	>500
D-04	3:1	70	30	1	125
D-05	3:1	60	40	1	80
D-06	3:1	50	50	1	30
D-07	3:1	40	>60	No transparency	
D-08	2:1	90	10	1	>500
D-09	2:1	80	20	1	>500
D-10	2:1	70	30	1	95
D-11	2:1	60	40	1	75
D-12	2:1	50	50	1	30
D-13	2:1	40	>60	No transparency	
D-14	1:1	90	10	1	>500
D-15	1:1	80	20	1	105
D-16	1:1	70	60	1	60
D-17	1:1	60	40	1	28
D-18	1:1	50	>50	No transparency	
D-19	1:2	90	10	1	>500
D-20	1:2	80	20	1	80
D-21	1:2	70	30	1	55
D-22	1:2	60	40	1	15
D-23	1:2	50	>50	No transparency	
D-24	1:0	90	>10	Thick mass obtained. No transparency	

[1] Mixture of surfactant (ST) and cosurfactant represented as STmix. % Of STmix and oil was calculated with respect to the total quantity of STmix and oil.

* Calculation of water composition was based on the total quantity of STmix and oil mixture and represented as parts.

4.7. Incorporation of drug into the microemulsion system:

4.7.1. Acyclovir:

Surfactant and cosurfactant mixture of different ratio (Km= 4:1, 3:1 and 2:1 for system A and Km=3:1, 2:1 and 1:1 for system B) was prepared. Labrafac was added into the preformed surfactant mixture (STmix) at 20:80 ratios. The known quantity of acyclovir was added into the mixture of labrafac and STmix with a constant stirring until the mixture become clear. The resultant microemulsion pre-concentrate was diluted by 100% with water. The mixture was gently shaken and kept at ambient temperature (25°C) to obtain a clear or translucent microemulsion. The different known quantity of acyclovir was added into the mixture of STmix and oil to get the final concentration of acyclovir in microemulsion to 0mg/ml, 5mg/ml, 10mg/ml, 15mg/ml and 20mg/ml.

To check any un-dissolved or precipitated drug in the microemulsion system, the concentration of was acyclovir was checked after 2 hours and after 3days. Briefly acyclovir loaded microemulsion was filtered through 0.45m membrane filter to separate any undissolved or precipitated drug. The amount of acyclovir in the resulting clear filtrate was estimated by UV spectrophotometer at 252nm after appropriate dilution with ethanol.

4.7.2. Efavirenz:

Surfactant and cosurfactant mixture of different ratio (Km= 4:1, 3:1 and 2:1 for system C and Km=3:1, 2:1 and 1:1 for system D) was prepared. Labrafil M 1944Cs was added into the preformed surfactant mixture (STmix) at 20:80 ratios. The known quantity of efavirenz was added into the mixture of labrafil and STmix with a constant stirring until the mixture become clear. The resultant microemulsion pre-concentrate was diluted by 100% with water. The mixture was gently shaken and kept at ambient temperature (25°C)

obtain a clear or translucent microemulsion. The different known quantity of efavirenz was added into the mixture of STmix and oil to get the final concentration of acyclovir in microemulsion was 0mg/ml, 10mg/ml, 20mg/ml, 30mg/ml and 40mg/ml.

To check any un-dissolved or precipitated drug in the microemulsion system, the concentration of efavirenz was checked after 2 hours and after 3days. Briefly efavirenz loaded microemulsion was filtered through 0.45m membrane filter to separate any un-dissolved or precipitated drug. The amount of efavirenz in the resulting clear filtrate was estimated by UV spectrophotometer at 247nm after appropriate dilution with methanol.

4.8. Results and Discussion:

The following Table 4.6 illustrates the solubility of acyclovir in different oil phase as well as in different surfactant (Table 4.7).

Table 4.6: Solubility of acyclovir and efavirenz in different oil

S. No	Name of the ingredient	Solubility (mg/ml)	
		Acyclovir	Efavirenz
1	Labrafac CC	**15.96**	85.64
2	Labrafac Lipo	12.39	105.01
3	Labrafac hydro	11.80	98.82
4	Benzyl alcohol	11.08	74.56
5	Sun flower oil	4.36	30.95
6	Soybean oil	5.95	84.65
7	Lauroglycol FCC	2.72	75.06
8	Miglyol 812	11.35	125.35
9	Labrafil M 1944 CS	7.37	**153.51**
10.	Labrafil M 2125 CS	8.25	89.24

Table 4.7: Solubility of acyclovir and efavirenz in different surfactant.

S. No	Name of the ingredient	Solubility (mg/ml)	
		Acyclovir	Efavirenz
1	Tween 80	**12.99**	55.10
2	Cremophor RH 40	3.94	**66.88**
3	Labrasol	**14.33**	**79.46**
4	SLS	3.53	17.78
5	Polaxomer	2.58	3.41
6	Propylene Glycol	**8.32**	**100.21**
7	PEG 400	2.68	95.62
8	Transcutol	4.65	**251.91**
9	Plurol Olique	**9.56**	16.78
10.	Triacetin	5.68	66.88

Form the Table 4.6 it was observed that the greater solubility of acyclovir was in labrafac and benzyl alcohol (as oil phase), in Labrasol and tween 80 (as surfactant) and in plurol olique and propylene glycol (as cosurfactant).

Similarly, from Table 4.7 it was also observed that efavirenz show maximum solubility in labrafil M1944 CS (as oil phase), Cremophor RH 40, Labrasol (as surfactant) and in transcutol, propylene glycol (as cosurfactant).

4.8.1 Interfacial tension measurements:

The mixture of oil and water forms a microemulsion only when interfacial tension (IFT) between the two phases attains very low values (tends to zero). Interfacial tension is a key factor for microemulsion formation and that the main role of COST is to reduce the interfacial tension. (J.H. Sculmann et al.1959).

4.8.1.1 Acyclovir:

4.8.1.1.1. Labrasol, plurol olique, labrafac and water system (System A):

As shown in Table 4.8, addition of COST upto 90% w/w reduced the interfacial tension of pure oil from 11.86 dyne/cm to 1.45 dyne/cm. Similarly, interfacial tension data for the

various oil-ST mixture revealed that the incorporation of ST led to a dramatic decrease in the interfacial tension which reached a minimum at 80% ST concentration. When the combination of both the ST (Labrasol ®) and COST (Plurol Olique) mixture (Km=4) was added into the oil phase (Labrafac®), the interfacial tension of the system reached minimum (tends to zero) at a concentration of 70%. It is evident from the Table 4.8 and Figure 4.1 that interfacial efficiency of the ST and COST mixture is better than ST or COST alone. However, the interfacial tension reduction in case of ST was found more pronounced than COST. It is also observed that the microemulsion can also be formed in presence of ST only (without COST) but high concentration of ST is needed to form the microemulsion than the ST+COST mixture. It could be suggested that the COST helped decrease the interfacial tension of the microemulsion system with a synergistic relationship existing between ST and COST.

Table 4.8: Effect of labrasol and / or plurol olique on interfacial tension

% of oil in the mixture	Interfacial Tension (dyne/Cm)				
	Oil: COST mixture	Oil: ST mixture	Oil: ST + COST (Km=4)	Oil: ST + COST (Km=3)	Oil: ST + COST (Km=2)
10	1.45 ± 0.10	0	0	0	0
20	1.54 ± 0.11	0	0	0	0.07 ±0.04
30	1.66 ±0.14	0.11 ± 0.04	0	0.08 ± 0.02	0.23 ±0.06
40	1.79 ± 0.12	0.23 ± 0.06	0.05 ± 0.01	0.10 ±0.02	0.44 ±0.08
50	2.53 ± 0.31	0.32 ± 0.08	0.07 ± 0.02	0.32 ±0.12	0.84 ± 0.11
60	3.40 ± 0.26	0.54 ± 0.05	0.28 ± 0.11	0.71 ±0.16	2.12 ± 0.41
70	4.12 ± 0.36	1.15 ± 0.12	0.65 ± 0.16	2.34 ±0.64	3.02 ± 0.26
80	4.78 ± 0.41	2.45 ± 0.63	2.18 ± 0.32	3.42 ±0.46	3.88 ±0.74
90	6.45 ± 0.64	3.72 ± 0.78	3.64 ± 0.46	4.56 ±0.72	4.87 ±0.89
100	11.86 ± 1.23	11.86 ± 1.23	11.86 ± 1.23	11.86 ± 1.23	11.86 ± 1.23

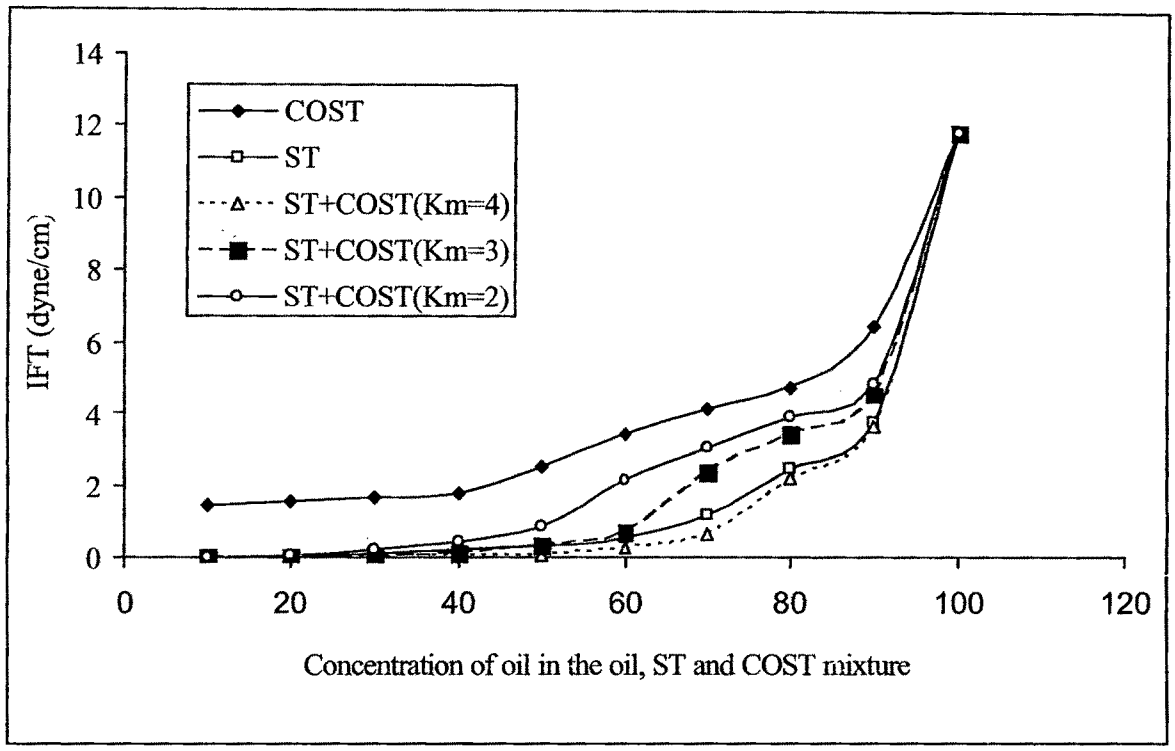


Figure 4.1: Effect on interfacial tension (IFT) of oil in presence of ST, COST and ST+ COST mixture (System A)

4.8.1.1 2. Tween 80, propylene glycol, labrafac and water system (System B)

As shown in Table 4.9 addition of propylene glycol upto 90% w/w reduced the interfacial tension of pure oil from 11.86 dyne/cm to 2.82 dyne/cm. Similarly, interfacial tension data for the various oil- surfactant (ST) mixture revealed that the incorporation of tween 80 led to a dramatic decrease in the interfacial tension which reached a minimum at 80% ST concentration. When the combination of both the ST (tween 80) and COST (propylene glycol) mixture (Km=3) was added into the oil phase (Labrafac), the interfacial tension of the system reaches minimum (tends to zero) at a concentration of 70%. Similarly, interfacial tension of the oil and ST mixture (ST + COST) was tends to zero at a concentration for ST mixture of 70% or 80% when Km = 2 or 1 respectively. It is evident from the Table 4.9 and Figure 4.2 that interfacial efficiency of the ST and COST mixture is better than ST or COST alone. However, the interfacial tension reduction in case of ST was found more pronounced than COST. It is also observed that

the microemulsion can also be formed in presence of ST only (without COST) but high concentration of ST is needed to form the microemulsion than the ST+COST mixture. It could be suggested that the COST helped decrease the interfacial tension of the microemulsion system with a synergistic relationship existing between ST and COST.

Table 4.9: Effect of tween 80 and/ or propylene glycol on interfacial tension

% of oil in the mixture	Interfacial Tension (dyne/Cm)				
	Oil: COST mixture	Oil: ST mixture	Oil: ST + COST (Km=3)	Oil: ST + COST (Km=2)	Oil: ST + COST (Km=1)
10	2.82 ±0.42	0	0	0	0.08 ±0.02
20	3.51 ± 0.34	0.04 ±0.02	0	0	0.12 ± 0.05
30	3.46 ± 0.25	0.16 ±0.04	0.03 ± 0.03	0.04 ± 0.03	0.24 ± 0.05
40	4.12 ± 0.49	0.46 ±0.13	0.16 ±0.06	0.14 ± 0.07	0.96 ± 0.08
50	6.21 ± 0.54	0.64 ±0.31	0.55 ± 0.22	0.58 ±0.12	1.24 ± 0.22
60	6.84 ± 0.85	1.85 ±0.53	1.14 ± 0.30	1.34 ±0.21	2.46 ± 0.44
70	8.12 ± 0.84	2.92 ±0.64	1.96 ± 0.42	2.42 ±0.32	3.64 ± 0.25
80	8.92 ± 0.64	3.75 ± 0.46	2.71 ±0.26	3.21 ±0.46	5.44 ± 0.74
90	9.23 ± 1.03	4.98 ± 0.68	3.92 ± 0.74	4.11 ± 0.68	6.82 ± 0.98
100	11.86 ± 1.23	11.86 ± 1.23	11.86 ± 1.23	11.86 ± 1.23	11.86 ± 1.23

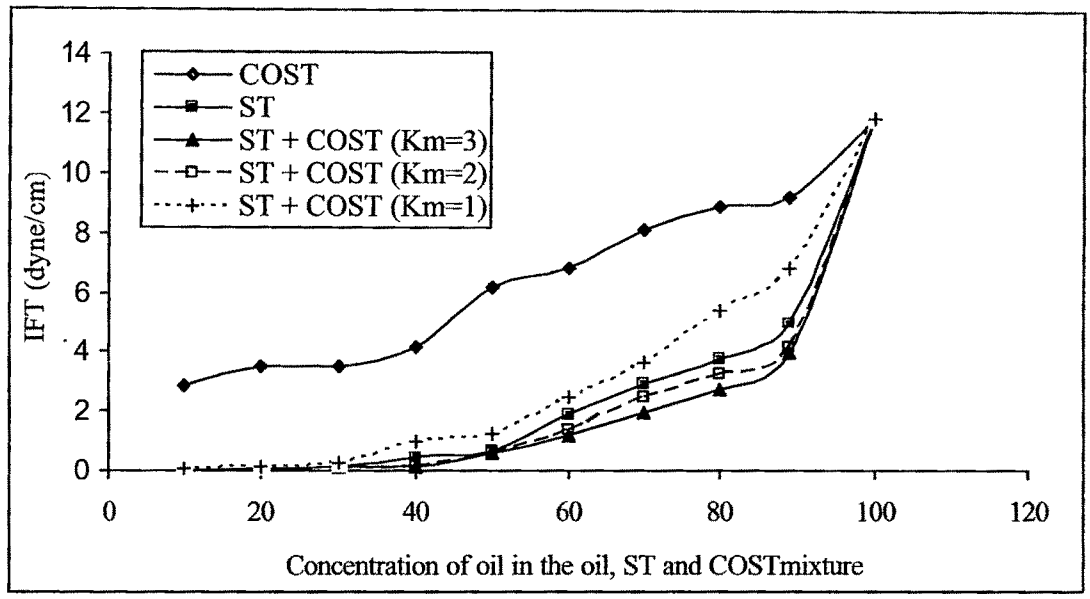


Figure 4.2: Effect on interfacial tension (IFT) of oil in presence of ST, COST and ST+ COST mixture (System B)

4.8.2. Efavirenz

4.8.2.1. Labrasol transcutol, labrafil M 1944 CS and water system (System C):

As shown in Table 4.10, addition of COST upto 90% w/w reduced the interfacial tension of pure oil from 9.46 dyne/cm to 2.25 dyne/cm. Similarly, interfacial tension data for the various oil-ST mixture revealed that the incorporation of ST led to a dramatic decrease in the interfacial tension which reached a minimum at 80% ST concentration. When the combination of both the ST (Labrasol) and COST (transcutol) mixture (Km=3) was added into the oil phase (Labrafil M 1944 CS), the interfacial tension of the system reaches minimum (tends to zero) at a concentration of 70%. It was evident from the Table 4.10 and Figure 4.3 that interfacial efficiency of the ST and COST mixture is better than ST or COST alone. However, the interfacial tension reduction in case of ST was found more pronounced than COST. It is also observed that the microemulsion can also be formed in presence of ST only (without COST) but high concentration of ST is needed to form the microemulsion than the ST+COST mixture. It could be suggested that the COST helped decrease the interfacial tension of the microemulsion system with a synergistic relationship existing between ST and COST.

Table 4.10: Effect of labrasol and/ or transcutol on interfacial tension

% of oil in the mixture	Interfacial Tension (dyne/Cm)				
	Oil: COST mixture	Oil: ST mixture	Oil: ST + COST (Km=3)	Oil: ST + COST (Km=2)	Oil: ST + COST (Km=1)
10	2.25	0	0	0	0
20	2.65	0	0	0	0.06
30	2.98	0.12	0.05	0	0.18
40	3.25	0.31	0.16	0.13	0.62
50	3.42	0.61	0.22	0.49	0.98
60	3.84	0.85	0.36	0.54	1.22
70	4.25	1.74	1.48	1.57	2.17
80	5.42	2.62	2.33	2.72	3.14
90	6.46	3.83	3.44	3.98	4.12
100	9.46	9.46	9.46	9.46	9.46

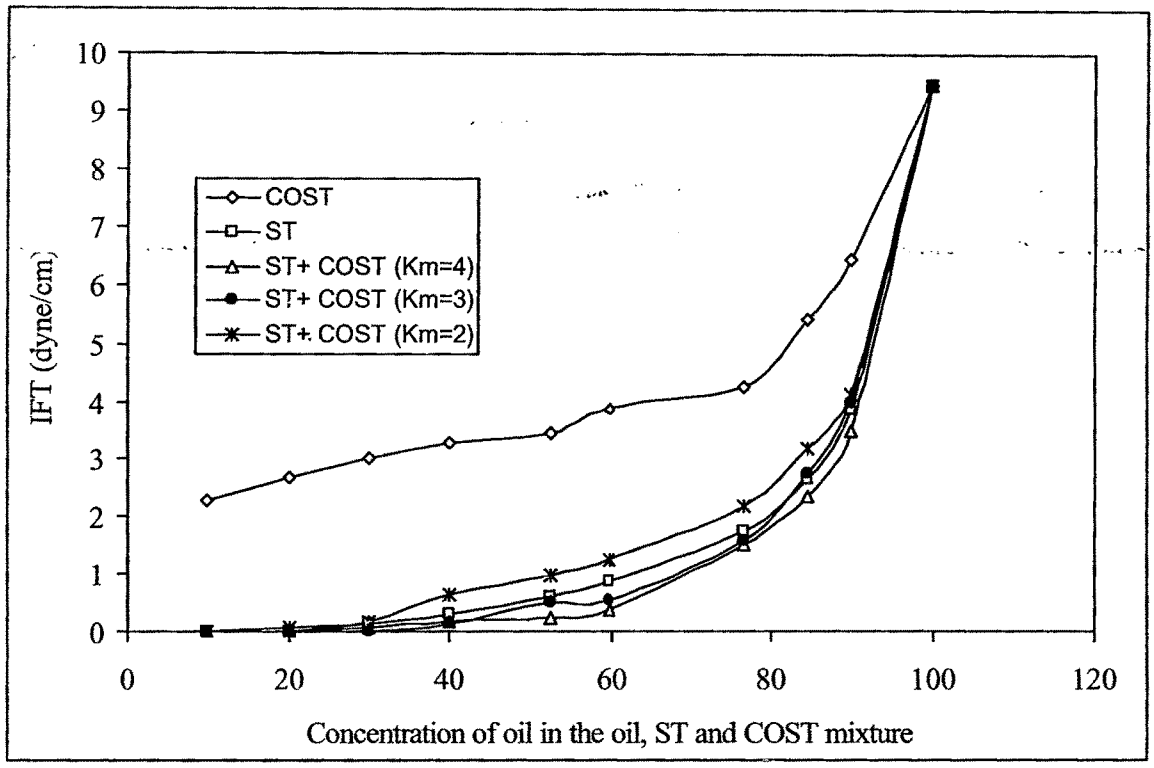
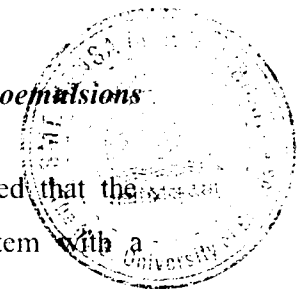


Figure 4.3: Effect on interfacial tension (IFT) of oil in presence of ST, COST and ST+ COST mixture (system C)

4.8.2.2. Cremophor RH 40, propylene glycol, labrafil M 1944C and water system (System D):

As shown in Table 4.11, addition of COST upto 90% w/w reduced the interfacial tension of pure oil from 9.46 dyne/cm to 2.14 dyne/cm. Similarly, interfacial tension data for the various oil-ST mixture revealed that the incorporation of ST led to a dramatic decrease in the interfacial tension which reached a minimum at 80% ST concentration. When the combination of both the ST (Labrasol) and COST (transcutol) mixture (Km=3) was added into the oil phase (Labrafil M 1944 CS), the interfacial tension of the system reaches minimum (tends to zero) at a concentration of 70%. It was evident from the Table 4.11 and Figure 4.4 that interfacial efficiency of the ST and COST mixture is better than ST or COST alone. However, the interfacial tension reduction in case of ST was found more pronounced than COST. It is also observed that the microemulsion can also be formed in presence of ST only (without COST) but high concentration of ST is needed to



form the microemulsion than the ST+COST mixture. It could be suggested that the COST helped decrease the interfacial tension of the microemulsion system with a synergistic relationship existing between ST and COST.

Table 4.11: Effect of cremophor RH40 and/ or propylene glycol on interfacial tension

% of oil in the mixture	Interfacial Tension (dyne/Cm)				
	Oil: COST mixture	Oil: ST mixture	Oil: ST + COST (Km=3)	Oil: ST + COST (Km=2)	Oil: ST + COST (Km=1)
10	2.14	0	0	0	0
20	2.62	0	0	0	0
30	3.45	0.16	0.08	0	0.22
40	3.67	0.27	0.12	0.18	0.34
50	3.84	0.58	0.46	0.56	1
60	3.98	1.46	1.09	1.42	1.85
70	4.25	1.84	1.64	1.98	2.12
80	5.24	2.48	2.13	2.42	2.68
90	7.45	3.27	2.78	2.87	3.47
100	9.46	9.46	9.46	9.46	9.46

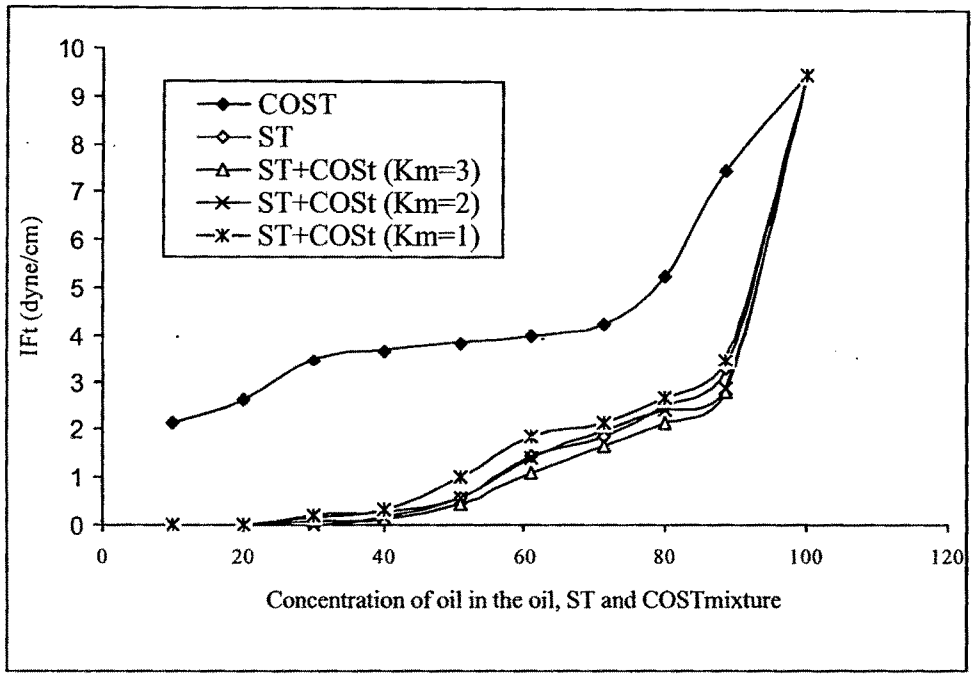


Figure 4.4: Effect on interfacial tension (IFT) of oil in presence of ST, COST and ST+ COST mixture (system D)

4.9. Preparation of microemulsion:

4.9.1. Acyclovir:

4.9.1.1. Labrasol, plurol olique, labrafac and water system (System A):

It was seen from the above Table 4.2 that ratio of ST to COST (Km) play a major role for the formation of microemulsion and also for the maximum uptake of water. From the above table it was also observed that maximum uptake of water takes place when percentage of labrafac is less. As percentage of labrafac increases the amount of water uptake was less, irrespective of the ratio of ST to COST. It was observed that there was no formation of microemulsion when Km < 1. Hence the further study was done for the system when Km >1:1 and other batch discarded.

The effect of Km and labrafac content on the maximum water uptake to form microemulsion was shown in Table 4.12. From this Table 4.12 it was observe that, 2:1 ratio of ST to COST accommodate more quantity of water as compared to other ratio for example 4:1 or 3:1. When STmix: labrafac was 90: 10, then maximum water uptake was 60%, 63.6% and 74% for Km 4:1, 3:1 and 2:1 respectively. Similarly, when STmix:

labrafac 80:20, then maximum water uptake was 51.4%, 58.6% and 69.6% for Km 4:1, 3:1 and 2:1 respectively and when STmix: labrafac was 70: 30, then maximum water uptake was 27.5%, 3.50% and 38.2% for Km 4:1, 3:1 and 2:1 respectively. And when STmix: labrafac was 60: 40, then maximum water uptake was 14.5%, 21.8% and 25.9% for Km 4:1, 3:1 and 2:1 respectively.

Table 4.12: Effect of ST to COST ratio and labrafac content on the % water uptake to form microemulsion (System A)

B. No	ST to COST ratio (Km)	Ratio of STmix: Labrafac	% Water range to form microemulsion	
			Minimum	Maximum
A-01	4:1	90:10	12.2	60
A-10	3:1	90:10	16.6	63.6
A-19	2:1	90:10	23.0	74
A-02	**4:1**	**80:20**	**9.9**	**51.4**
A-11	**3:1**	**80:20**	**14.5**	**58.6**
A-20	**2:1**	**80:20**	**19.3**	**69.6**
A-03	4:1	70:30	9.1	27.5
A-12	3:1	70:30	12.2	30.5
A-21	2:1	70:30	16.6	38.2
A-04	4:1	60:40	7.4	14.5
A-13	3:1	60:40	10.7	21.8
A-22	2:1	60:40	12.2	25.9

It was also seen from the Table 4.12 that minimum quantity of water required for the formation of microemulsion was larger when Km was 2:1 as compared when Km was 3:1 or 4:1. For the formation of microemulsion, when ratio of STmix: labrafac was 90:10, then minimum water required for formation of microemulsion was 12.2%, 16.6% and 23% at Km= 4:1, 3:1 and 2:1 respectively Similarly, for 80:20 ratio of STmix : labrafac minimum 9.9%, 14.5% & 19.3% of water was required at Km= 4:1, 3:1 & 2:1 respectively and for 70 30 ratio of STmix : labrafac minimum 9.1%, 12.2% and 16.6%of water required at Km= 4:1, 3:1 & 2:1 respectively and for 60 40 ratio of STmix : labrafac minimum 7.4%, 10.7% and 12.2%of water required at Km= 4:1, 3:1 & 2:1

respectively. Although when STmix: labrafac ratio was 90:10, which can accommodate maximum quantity of water irrespective of the Km, but in this formulation it was difficult to get the concentration of drug 10mg/ml.due to less quantity of oil phase. Besides surfactant concentration will be more as compared to when STmix: labrafac was 80:20. Based on the above observation, we finalized the ratio of ST mix: oil was 80:20 or in other words, oil/STmix=0.25.

4.9.1.2. Tween 80, propylene glycol, labrafac and water system (System B):

It was seen from the above Table 4.3 that ratio of ST to COST (Km) play a major role for the formation of microemulsion and also for the maximum dilution with water. From the above Table 4.3 it was also observed that maximum uptake of water takes place when percentage of labrafac is less. As percentage of labrafac increases the amount of water uptake was less, irrespective of the ratio of ST to COST. It was observed that there was no formation of microemulsion when 4:1 = Km < 1:2 and no formation of microemulsion takes place when no cosurfactant was added in the system. Hence the further study was done for the system when 4:1> Km> 1:2 and other batch discarded.

Table 4.13: Effect of ST to COST ratio and labrafac content on the % water uptake to form microemulsion (System B)

B. No	ST to COST ratio (Km)	Ratio of STmix: Labrafac	Maximum % of water retains transparency
B-02	3:1	90:10	~100
B-11	2:1	90:10	~100
B-20	1:1	90:10	~100
B-03	3:1	**80:20**	**~100**
B-12	2:1	**80:20**	**~100**
B-21	1:1	**80:20**	**61**
B-04	3:1	70:30	58
B-13	2:1	70:30	44
B-22	1:1	70:30	35
B-05	3:1	60:40	45
B-14	2:1	60:40	32
B-23	1:1	60:40	16

The effect of Km and labrafac content on the maximum water uptake to form microemulsion was shown in Table 4.13. From this Table 4.13 it was observed that, 3:1 ratio of ST to COST accommodate more quantity of water as compared to other ratio for example 2:1 or 1:1. When ratio of STmix: labrafac was 90: 10, then upon dilution (100times) with water the system retains their transparency irrespective of Km.. But, when ratio of STmix: labrafac 80:20, on 100 times dilution with water, system retains it transparency for Km= 3:1 and 2:1 ratio only but at Km= 1:1, when % of water > 61% it becomes turbid. When ratio of STmix: labrafac was 70: 30, then transparency retains upto 58%, 44% and 35% of water for Km 3:1, 2:1 and 1: 1 respectively. And when ratio of STmix: labrafac was 60: 40, then maximum water uptake was 45%, 32% and 16% for Km 3:1, 2:1 and 1:1 respectively.

4.9.2. Efavirenz

4.9.2.1. Labrasol transcutol, labrafil M 1944 CS and water system (System C):

Here also, ratio of ST to COST (Km) plays a major role for the formation of microemulsion and also for the maximum uptake of water. From the above Table 4.4 it was also observed that upon dilution with water, microemulsion system retains their transparency when percentage of labrafil M 1944 CS is less. As percentage of labrafil M1944 CS increases the amount of water uptake was less, irrespective of the ratio of ST to COST. It was observed that there was no formation of microemulsion when Km < 1:2 or when no cosurfactant was added in the system. Hence the further study was done for the system when 4:1= Km > 1:2 and other batch was discarded.

Table 4.14: Effect of ST to COST ratio and labrafac content on the % water uptake to form microemulsion (System C)

B. No	ST to COST ratio (Km)	Ratio of STmix: Labrafil M1944 CS	Maximum % of water retains transparency
C-01	4:1	90:10	~100
C-08	3:1	90:10	~100
C-14	2:1	90:10	~100
C-20	1:1	90:10	~100
C-02	4:1	**80:20**	**~100**
C-09	3:1	**80:20**	**~100**
C-15	2:1	**80:20**	**~100**
C-21	1:1	**80:20**	**54**
C-03	4:1	70:30	69
C-10	3:1	70:30	58
C-16	2:1	70:30	40
C-22	1:1	70:30	25
C-04	4:1	60:40	61
C-11	3:1	60:40	48
C-17	2:1	60:40	29
C-23	1:1	60:40	13

The effect of Km and labrafil M 1944 CS content on the maximum dilution with water was shown in Table 4.14. From this Table 4.14 it was observed that, 4:1 ratio of ST to COST accommodate more quantity of water as compared to other ratio for example 3:1 or 2:1 or 1:1. When ratio of STmix: labrafil M 1944CS was 90: 10, then upon dilution (100times) with water the system retains their transparency irrespective of Km.. But, when ratio of STmix: labrafac 80:20, on 100 times dilution with water, system retains it transparency for Km= 4:1, 3:1 and 2:1 ratio only but at Km= 1:1, when % of water > 54% it becomes turbid. When ratio of STmix: labrafil M 1044 CS was 70: 30, then transparency retains upto 69%, 58%, 40% and 25% of water for Km= 4:1, 3:1, 2:1 and 1:

1 respectively. And when ratio of STmix: labrafil M 1944 CS was 60: 40, then maximum water uptake was 61%, 48%, 29% and 13% for Km 4:1, 3:1, 2:1 and 1:1 respectively.

4.9.2.2. Cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (System D):

Here also, ratio of ST to COST (Km) plays a major role for the formation of microemulsion and also for the maximum uptake of water. From the above Table 4.5 it was also observed that upon dilution with water, microemulsion system retains their transparency when percentage of labrafil M 1944 CS is less. As percentage of labrafil M1944 CS increases the amount of water uptake was less, irrespective of the ratio of ST to COST. It was observed that there was no formation of microemulsion when 4:1< Km < 1:2 or when no cosurfactant was added in the system. Hence the further study was done for the system when 3:1= Km > 1:2 and other batch discarded.

Table 4.15: Effect of ST to COST ratio and labrafac content on the % water uptake to form microemulsion (System D)

B. No	ST to COST ratio (Km)	Ratio of STmix: Labrafil M1944 CS	Maximum % of water retains transparency
D-02	3:1	90:10	~100
D-08	2:1	90:10	~100
D-14	1:1	90:10	~100
D-19	1:2	90:10	~ 100
D-03	3:1	**80:20**	**~ 100**
D-09	2:1	**80:20**	**~ 100**
D-15	1:1	**80:20**	**51**
D-20	1:2	**80:20**	**44**
D-04	3:1	70:30	55
D-10	2:1	70:30	48
D-16	1:1	70:30	37
D-21	1:2	70:30	35
D-05	3:1	60:40	44

Cont....

D-11	2:1	60:40	42
D-17	1:1	60:40	21
D-22	1:2	60:40	13

The effect of Km and labrafil M 1944 CS content on the maximum dilution with water was shown in Table 4.15. From this Table 4.15 it was observe that, 3:1 ratio of ST to COST accommodate more quantity of water as compared to other ratio for example 2:1 or 1:1 or 1:2. When ratio of STmix: labrafil M 1944CS was 90: 10, then upon dilution (100times) with water the system retains their transparency irrespective of Km.. But, when ratio of STmix: labrafac 80:20, on 100 times dilution with water, system retains it transparency for Km= 3:1 and 2:1 ratio only but at Km= 1:1 & 1:2, the system become turbid when % of water > 51% and >44% respectively. When ratio of STmix: labrafil M 1044 CS was 70: 30, then transparency retains upto 55%, 48%, 37% and 35% of water for Km= 3:1, 2:1, 1:1 and 1: 2 respectively. And when ratio of STmix: labrafil M 1944 CS was 60: 40, then maximum water uptake was 44%, 42%, 21% and 13% for Km 3:1, 2:1, 1:1 and 1:2 respectively.

4.10. Phase diagram:

4.10.1. Acyclovir

Pseudo-ternary phase diagram of the investigated quaternary system labrasol, plurol olique, labrafac and water is presented in Figure 4.5 to Figure 4.7. Formation of microemulsion systems (the shaded area) was observed at room temperature. Phase behavior investigations of this system demonstrated the suitable approach to determine the water phase, oil phase, ST and COST concentration for which the transparent, one phase low-viscous microemulsion system was formed. During the addition of water in the selected oily mixtures there was a continuous transition from oil-rich systems (top side of the phase diagram) to water-rich system (left side of the phase diagram).

4.10.1.1. Labrasol, plurol olique, labrafac and water system (System A)

The obtained results show that the maximum solubilization of water was achieved in oil/surfactant/cosurfactant mixture when oil phase corresponds to < 20%. Reduction in ST content when oil phase contribute > 20% of the oil/ST/COST mixture showed lesser ability to solubilize the water phase.

The larger zone of microemulsion was found for the ratio of surfactant to cosurfactant 3:1,2:1 as compared 4:1. The pseudo-ternary phase diagrams were not constructed for the ratio of 1:1 or 1:2, as there was no transparency observed.

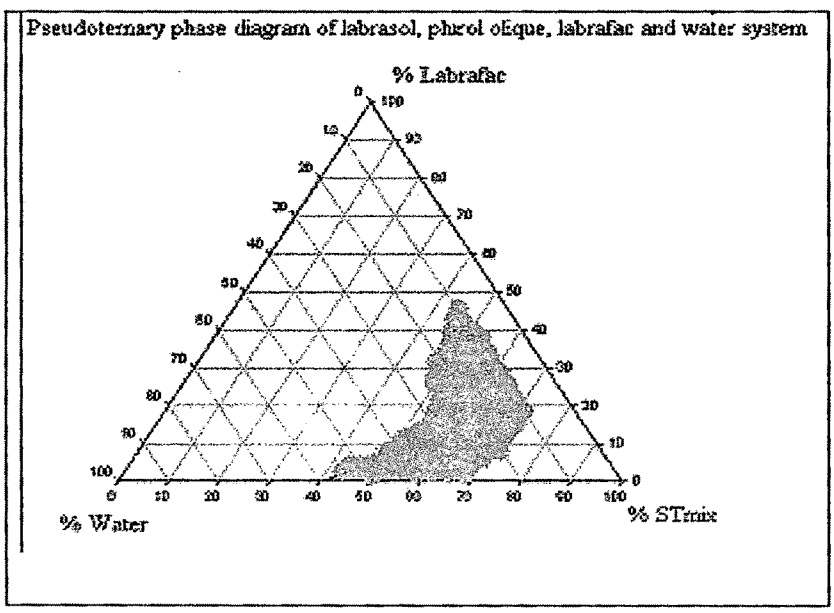


Figure 4.5: Pseudo-ternary phase diagram of labrasol, plurol olique, labrafac and water system (Km=4:1)

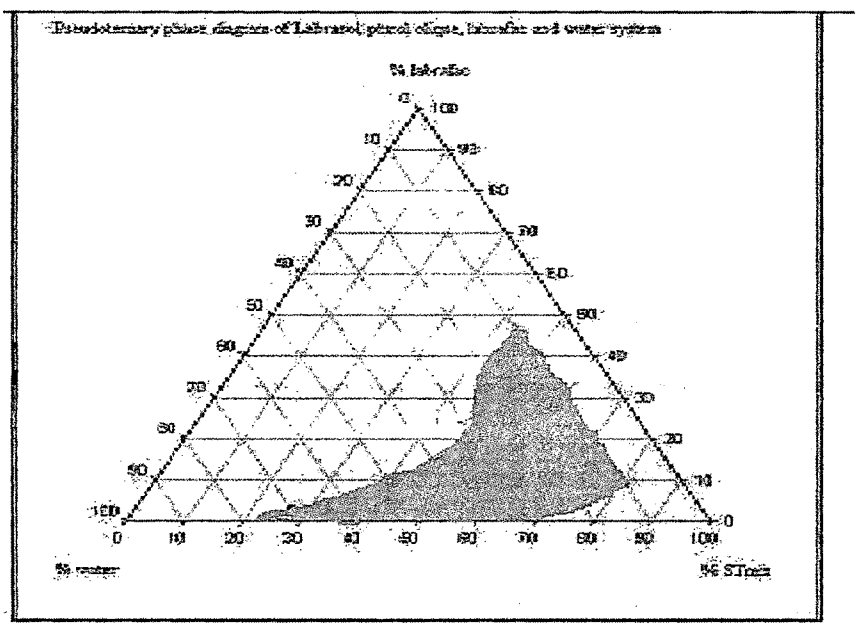


Figure 4.6: Pseudo-ternary phase diagram of labrasol, plurol olique, labrafac and water system (Km=3:1)

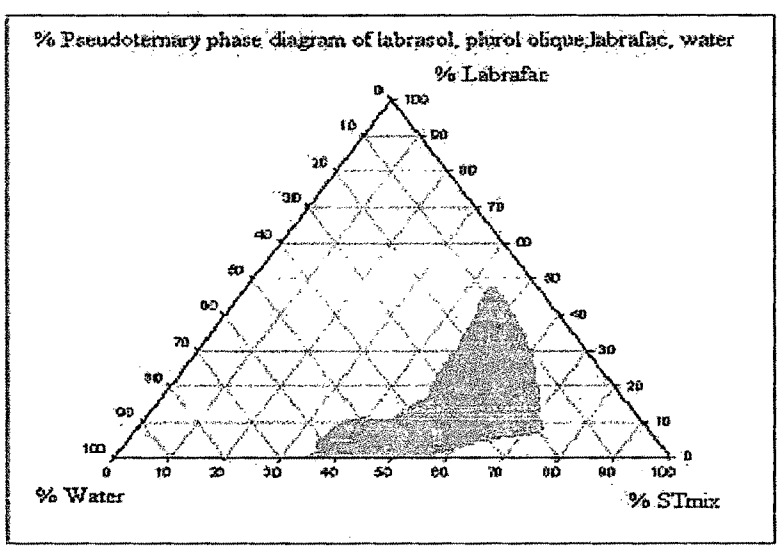


Figure 4.7. Pseudo-ternary phase diagram of labrasol, plurol olique, labrafac and water system (Km=2:1)

4.10.1.2. Tween 80, propylene glycol, labrafac and water system (System B):

The obtained results show that the maximum solubilization of water was achieved in oil/surfactant/cosurfactant mixture when oil phase corresponds to < 20%. Reduction in surfactant content or when oil phase contribute > 20% of the total oil and STmix showed lesser ability to retain its transparency upon dilution with water.

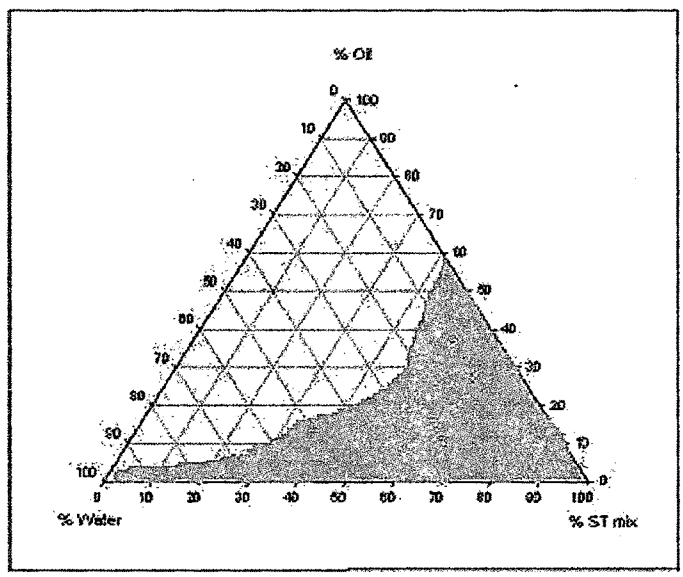


Figure 4.8 : Pseudo-ternary phase diagram of tween 80, propylene glycol, labrafac and water system (Km=3 :1)

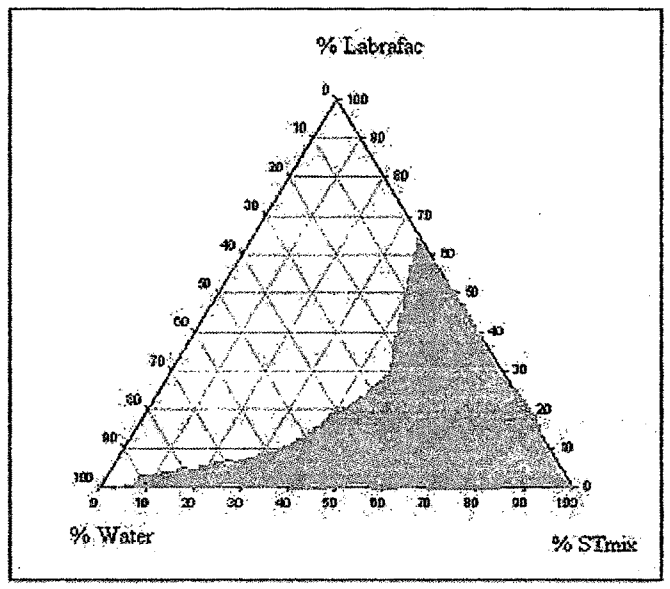


Figure 4.9: Pseudo-ternary phase diagram of tween 80, propylene glycol, labrafac and water system (Km=2:1)

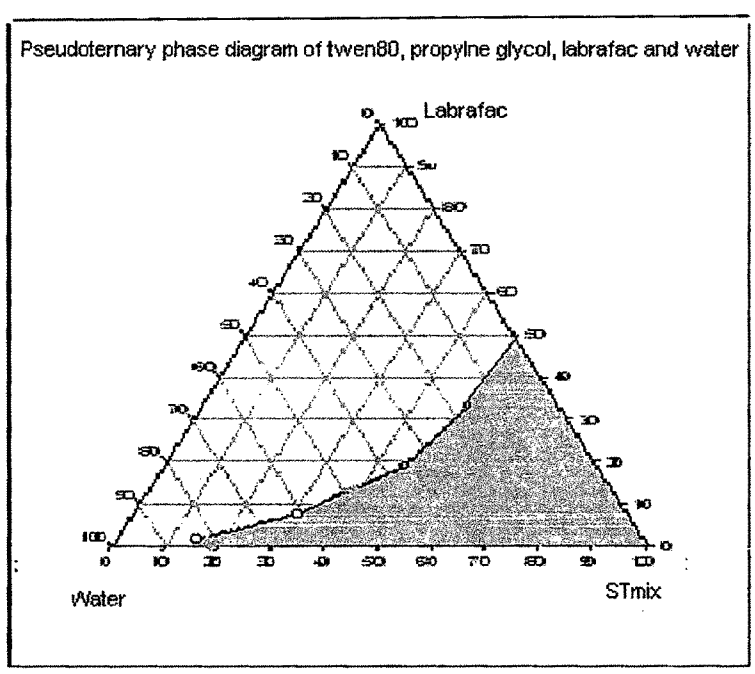


Figure 4.10 : Pseudo-ternary phase diagram of tween 80, propylene glycol, labrafac and water system (Km=1:1)

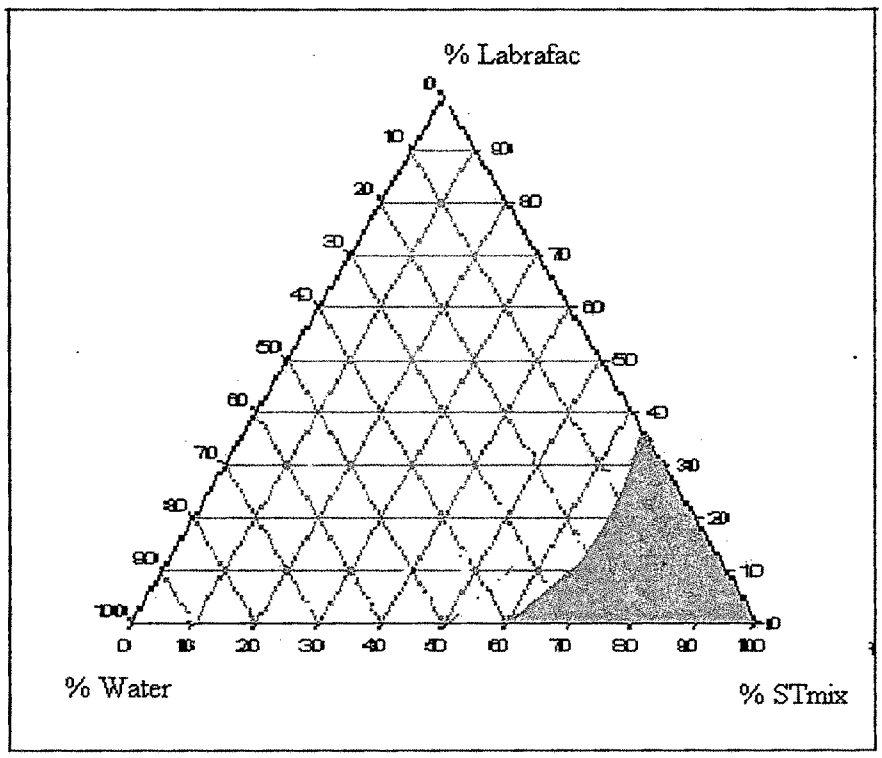


Figure 4.11: Pseudo-ternary phase diagram of tween 80, propylene glycol, labrafac and water system (Km=1:2)

Figure 4.8 to Figure 4.11 shows the pseudo-ternary phase diagram for Tween 80, Propylene glycol, labrafac and water system at different ratio of surfactant to cosurfactant (km). The similar microemulsion zone was found when Km is either 3:1 or 2:1., which are greater as compared to when Km=1:1 or 1:2. The pseudo-ternary phase diagrams were not constructed for the Km= 1:0 (i.e. no cosurfactant), as there was no transparency observed for any percentage of oil incorporation. Beside this, above Km=3:1, there was very viscous mass was found and was not studied further. As there was similar microemulsion was observed for both Km= 3:1 or 2:1, the Km=2:1 was fixed for further study as it consist of less quantity of surfactants.

4.10.2. Efavirenz

4.10.2.1. Labrasol, transcutol, labrafil M 1944 CS and water system (System C):

The obtained results show that the maximum solubilization of water was achieved in oil/surfactant/cosurfactant mixture when oil phase corresponds to < 20%. Reduction in surfactant content or when oil phase contribute > 20% of the total oil and STmix showed lesser ability to retain its transparency upon dilution with water.

Figure 4.12 to Figure 4.15 shows the pseudo-ternary phase diagram for labrasol, transcutol, labrafil M 1944 CS and water system at the different ratio of surfactant to cosurfactant (km). The greater microemulsion zone was found when Km is either 4:1 or 3:1. as compared to when Km=2:1 or 1:1 or 1:2. The pseudo-ternary phase diagrams were not constructed for the Km= 1:0 (i.e. no cosurfactant), as there was no transparency observed for any percentage of oil incorporation.

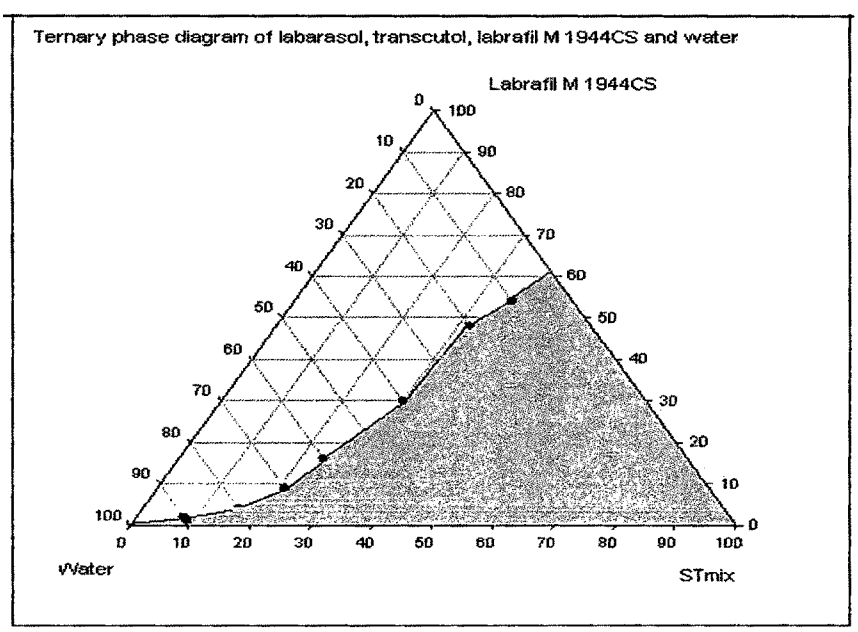


Figure 4.12: Pseudo-ternary phase diagram of labrasol, transcutol, labrafil M 1944CS and water system (Km=4:1)

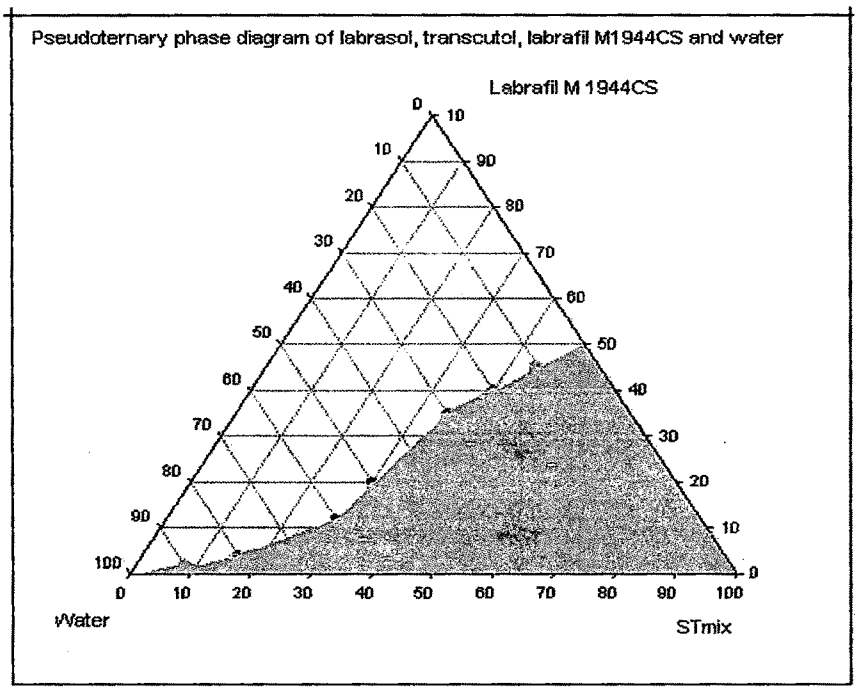


Figure 4.13: Pseudo-ternary phase diagram of labrasol, transcutol, labrafil M 1944CS and water system (Km=3:1)

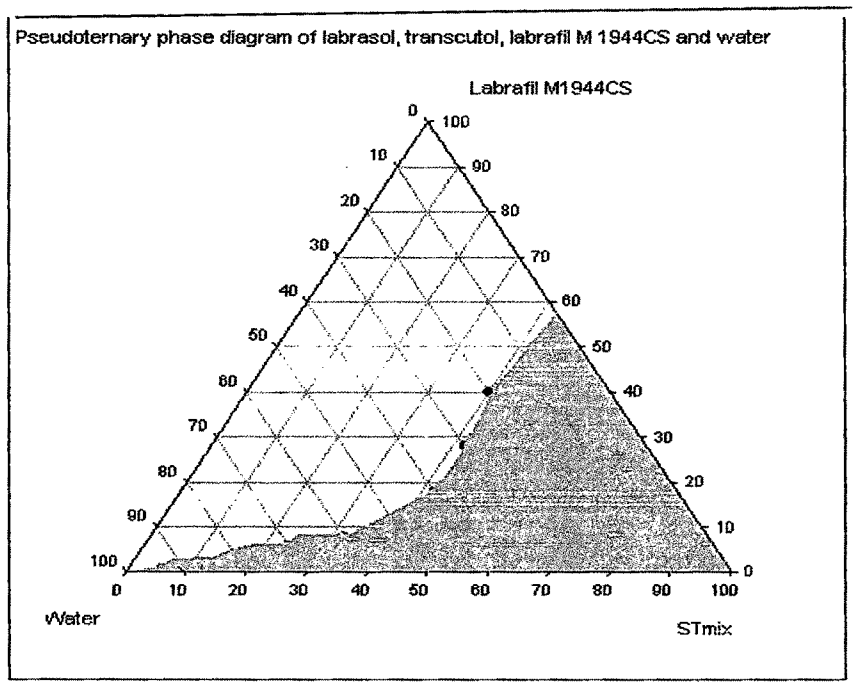


Figure 4.14: Pseudo-ternary phase diagram of labrasol, transcutol, labrafil M 1944CS and water system (Km=2:1)

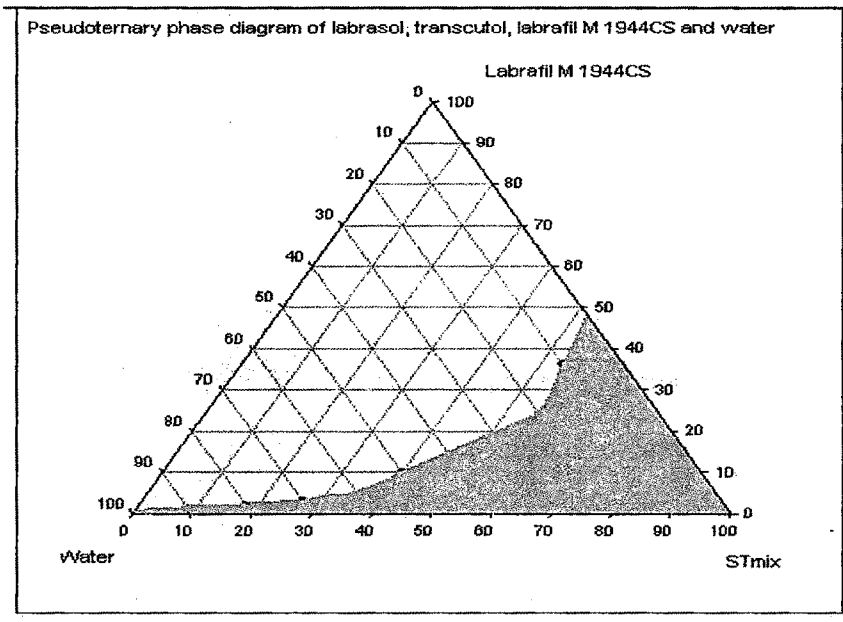


Figure 4. 15: Pseudo-ternary phase diagram of labrasol, transcutol, labrafil M 1944CS and water system (Km=1:1)

4.10.2.2. Cremophor RH 40, propylene glycol, labrafil M 1944 CS and water system (System D).

The results obtained show that the maximum solubilization of water was achieved in oil/surfactant/cosurfactant mixture when oil phase corresponds to < 20%. Reduction in surfactant content or when oil phase contribute > 20% of the total oil and STmix showed lesser ability to retain its transparency upon dilution with water.

Figure 4.16 to Figure 4.19 shows the pseudo-ternary phase diagram for cremophor RH 40, propylene glycol, labrafil M 1944 CS and water system at the different ratio of surfactant to cosurfactant (km). The greater microemulsion zone was found when Km is either 3:1 or 2:1, as compared to when Km=1:1 or 1:2. The pseudo-ternary phase diagrams were not constructed for the Km= 4:1 or 1:0 (i.e. no cosurfactant), as there was no transparency observed for any percentage of oil incorporation.

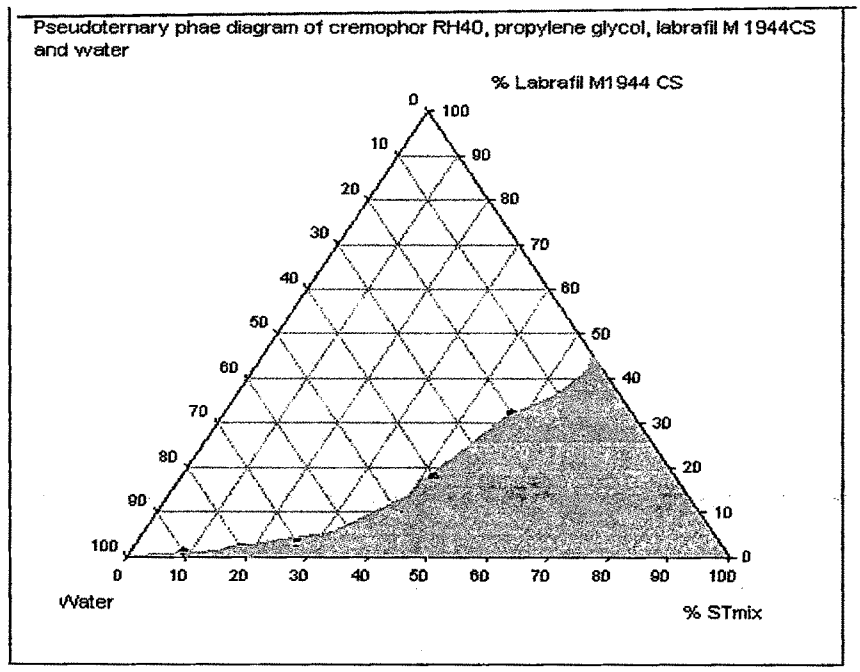


Figure 4.16: Pseudo-ternary phase diagram of Cremophor RH 40, propylene glycol, labrafil M 1944 CS and water system. (Km=3:1)

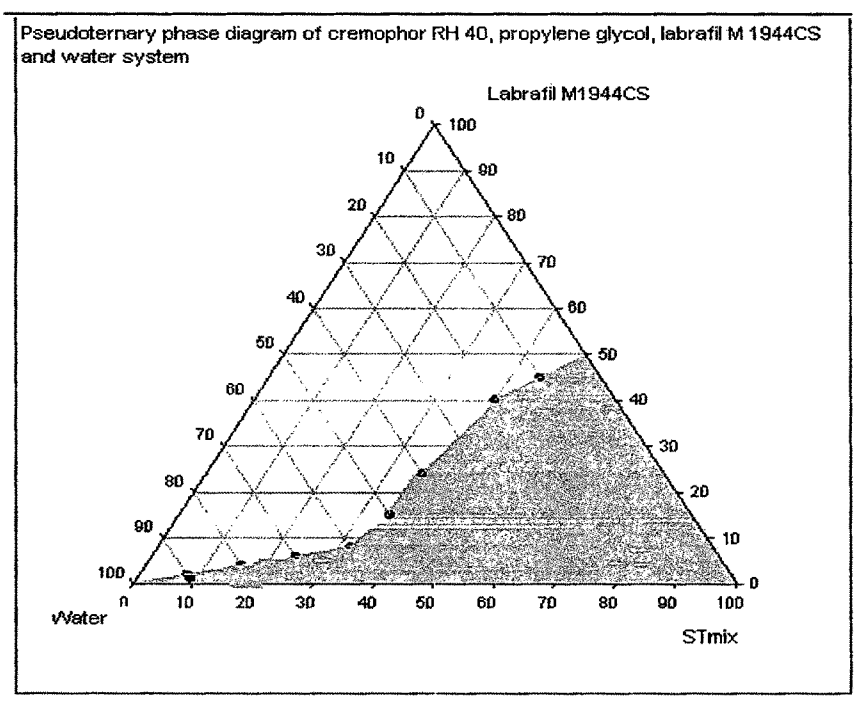


Figure 4.17: Pseudo-ternary phase diagram of Cremophor RH 40, propylene glycol, labrafil M 1944 CS and water system (Km=2:1)

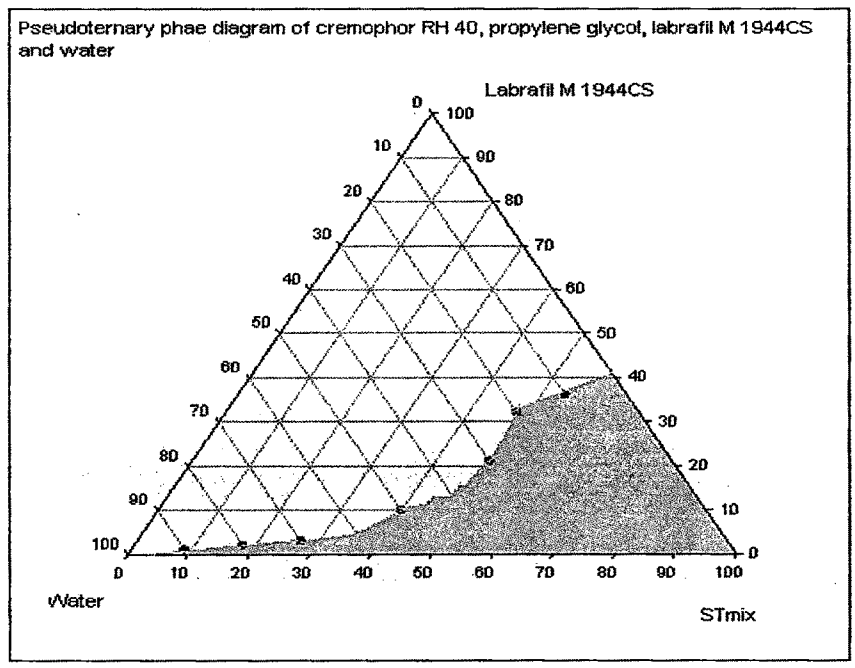


Figure 4.18: Pseudo-ternary phase diagram of Cremophor RH 40, propylene glycol, labrafil M 1944 CS and water system (Km=11)

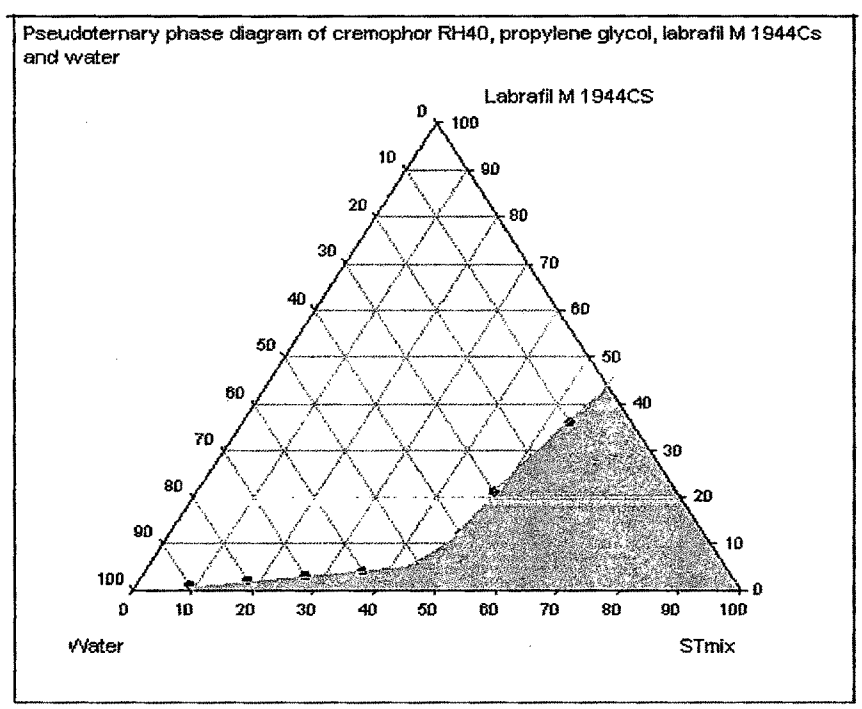


Figure 4.19: Pseudo-ternary phase diagram of Cremophor RH 40, propylene glycol, labrafil M 1944 CS and water system (Km=1:2)

4.11. Incorporation of drug into the microemulsion system:

4.11.1. Acyclovir:

The concentration of acyclovir was after 2hours of preparation and after 3 days of preparations was represented in Table 4.16. From the Table 4.16 it was observed that when acyclovir was loaded upto 10mg/ml, then there was no precipitation of the drug even after 3days from preparation of microemulsion. But when acyclovir was loaded as 15mg/ml, precipitation of the drug was observed after 3 days from preparation which was separated out during filtration process. When the drug load was 20mg/ml, some un-dissolved drug was still in the system which was separated by filtration and assay value of acyclovir was found 65-70% and 71 – 79% for system A and system B respectively, after 2 hours from preparation. The assay of acyclovir was found in the range of 61-64% and 66 – 75% for system A and system B respectively, after 3days of preparation. It is indicated that the excess amount of drug that existed in the interface of the oil–surfactant mixture of microemulsion preparation was released to the aqueous phase and grew the crystal and/or precipitate of acyclovir in the course of time. After the filtration of precipitates through the membrane filter (0.45 mm), the excess amount of surfactant did not affect the solubility of acyclovir in the acyclovir-loaded microemulsions.

Table 4.16: Concentration of acyclovir in the different microemulsion system at different Km after 2 hours and after 3 days from preparation.

Concentration of acyclovir (%)	System	Km	Acyclovir added (mg/ml)			
			5	10	15	20
After 2 Hours	A	4:1	99.6	99.5	95.3	72.3
		3:1	97.8	98.4	94.2	70.5
		2:1	98.8	98.2	90.4	65.2
After 3days	A	4:1	99.4	98.9	88.3	64.6
		3:1	98.2	99.1	84.2	61.6
		2:1	98.4	98.5	76.4	62.4

		3:1	99.4	99.7	95.2	79.6
After 2 Hours	B	2:1	98.4	98.7	93.2	74.2
		1:1	99.9	98.9	91.4	71.2
		3:1	99.3	99.1	84.3	75.3
After 3days	B	2:1	98.6	98.2	81.3	71.2
		1:1	99.8	98.3	79.3	66.9

4.11.2. Efavirenz:

The concentration of efavirenz after 2hours of preparation and after 3 days of preparations was represented in Table 4.17. From the Table 4.17 it was observed that when efavirenz was loaded upto 20mg/ml, then there was no precipitation of the drug even after 3days from preparation of microemulsion. But when efavirenz was loaded as 30mg/ml, precipitation of the drug was observed after 3 days from preparation which was separated out during filtration process. When the drug load was 40mg/ml, some un-dissolved drug was still in the system which was separated by filtration and assay value of efavirenz was found to be 88-93% and 89 -92% for system C and system D respectively, after 2 hours from preparation. The assay of efavirenz was found in the range of 80-83% and 83 -85% for system C and system D respectively, after 3days of preparation. It is indicated that the excess amount of drug that existed in the interface of the oil–surfactant mixture of microemulsion preparation was released to the aqueous phase and grew the crystal and/or precipitate of efavirenz in the course of time. After the filtration of precipitates through the membrane filter (0.45 mm), the excess amount of surfactant did not affect the solubility of efavirenz in the efavirenz-loaded microemulsions.

Table 4.17: Concentration of efavirenz in the different microemulsion system at different Km after 2 hours and after 3 days from preparation.

Concentration of efavirenz (%)	System	Km	Efavirenz added (mg/ml)			
			10	20	30	40
		4:1	99.4	101.3	101.4	89.4
After 2 Hours	C	3:1	98.9	99.7	97.6	88.5
		2:1	98.7	98.3	98.2	93.4

		4:1	99.6	99.3	94.6	81.4
After 3days	C	3:1	98.6	101.2	95.2	83.5
		2:1	99.1	102.2	93.7	80.4
		3:1	99.6	99.5	98.6	91.1
After 2 Hours	D	2:1	99.3	102.4	99.2	89.5
		1:1	98.7	103.2	100.8	92.4
		3:1	99.4	101.2	94.6	85.6
After 3days	D	2:1	100.2	99.8	92.5	83.4
		1:1	98.7	98.6	94.1	85.6

4.12. Conclusion:

4.12.1 Acyclovir:

Taking together all the above observations the (i) labrasol, plurol olique, labrafac and water system (system A) at surfactant to cosurfactant ratio 4:1, 3:1 and 2:1 (ii) Tween 80, propylene glycol, labrafac and water system at surfactant to cosurfactant ration 3:1, 2:1 and 1:1 was selected. In all the cases quantity of acyclovir was fixed and that is 10mg/ml

4.12.2 Efavirenz:

Taking together all the observations the (i) labrasol, transcutol, labrafil M1944CS and water system at surfactant to cosurfactant ratio 4:1, 3:1 and 2:1 (ii) Cremophor RH 40, propylene glycol, labrafil M1944CS and water system at surfactant to cosurfactant ratio a3:1, 2:1 and 1:1 was selected. In al the cases the quantity of efavirenz was fixed and that was20mg/ml.

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