Chapter 5

Microemulsion characterisations

5.1. Introduction:

In spite of many difficulties, it is essential to characterize the microstructure of a microemulsion but for successful commercial exploitation, it is essential to characterize the microstructure of a microemulsion. Physical and chemical characteristics of microemulsion influence their behavior *in vitro* and *in vivo*. Physical and chemical characterizations are very important for a meaningful comparison of different microemulsion preparation or different bathes prepared according to the same protocols. Biological evaluation helps to ensure safety of use in humans. As a rule, combination of various characterization methods is used, as none of the existing technique alone is able to describe microemulsion adequately. The various technique used in characterization have been extensively discussed previously. (Section: Literature review). For characterization of microemulsion we evaluate electrical conductivity, viscosity, refractive index, % transmittance, particle size and shape, polydispersity, zeta potential etc.

5.2 Experimental:

5.2.1. Apparatus: Conductometer (CM 180 conductivity meter, Elco, India), Brookefield LVDV 111 + CP viscometer (Mfg: Brookefield, USA), Zetasizer H_{SA} 3000(Malvern Instruments Ltd., Malvern, UK). Centrifuge (Remi Laboratories, Mumbai, India,) Refractometer (Abbe refractometer) pH meter (Elco, India) Transmission electron microscopy (TEM) (HITACHI H- 600 Electron Microscope),

5.2.2. Selection of microemulsion formulations:

5.2.2.1. Acyclovir:

From the pre-formulation study and phase diagram study, different microemulsion formulation was selected (Table 5.1 and Table 5.2) for further study. Microemulsion was prepared by simple mixing as described earlier. In all the cases the acyclovir was dissolved into the oil and surfactant mixture to get the final concentration of drug in microemulsion was 10mg/ml. All the samples were prepared 48h before investigations for distribution of oil, water and surfactant micelles to attain the thermodynamic equilibrium and were stored at room temperature. These batches were speculated to give

reasonably different types of colloidal microstructure, which could be confirmed, by electro-conductivity and viscosity.

5.2.2.1. 1.Detailed studies on Labrasol, plurol olique, labrafac and water (System A): From the constructed pseudo-ternary phase diagram of Labrasol / Labrafac / Plurol Olique/water, labrafac: STmix ratio was selected as 0.25 for further investigation. From the phase diagram study it was clear that the maximum solubilization of water was achieved when oil phase corresponds to < 20% irrespective of the ST to COST ratio (Km). Reduction in STmix content or when oil phase contribute > 20% of the oil and STmix showed lesser ability to solubilize the water phase. Also we have investigated the structural changes (for different ratio of surfactant to cosurfactant) in the microemulsion preconcentrate (A-MEP) with increasing the water content at constant ratio of labrafac and STmix and that is 0.25, in order to find out whether there is any relationship exists between the electro conductivity and viscosity in the microstructure. From the above investigations, six potential microemulsion vehicles (ME1 to ME6) were prepared (Table 5.1) to check the effect of concentration of surfactant and cosurfactant, ratio of surfactant to cosurfactant and concentration of labrafac content on physicochemical properties of microemulsion. At ME1, ME2 and ME3, total % of STmix was constant and that is 60% but surfactant to cosurfactant ratio was varied. In these formulation % of labrafac and water was kept at 15% and 25% respectively. Similarly, another three-microemulsion formulation (ME4, ME5 and ME6) was prepared by keeping the concentration of STmix 50% constant but ratio of surfactant to cosurfactant was varied. Similarly here also % of labrafac and water was constant and that is 12% and 40% respectively. In all the cases oil: STmix (0.25) were kept constant.

Table 5.1: Microemulsions vehicle composition (%w/w) at différent ST to COST ratio (Km) (System A)

Batch No	ST to COST ratio	Labrasol	Plurol olique	Labrafac	Water
ME1	4 :1	48	12	15	25
ME2	3 :1	45	15	15	25
ME3	2 :1	40	20	15	25
ME4	4 :1	32	8	10	50
ME5	3 :1	30	10	10	50
ME6	2 :1	26.7	13.3	10	50

5.2.2.1. 2. Detailed studies on Tween 80, propylene glycol, labrafac and water system (System B):

From the constructed pseudo-ternary phase diagram of tween 80 /propylene glycol /Labrafac/water system, labrafac: STmix ratio was selected as 0.25 for further investigation. From the phase diagram study it was clear that the maximum solubilization of water was achieved when oil phase corresponds to < 20% irrespective of the ST to COST ratio (Km). Reduction in STmix content or when oil phase contribute > 20% of the oil and STmix showed lesser ability to solubilize the water phase. Also we have investigated the structural changes in the microemulsion pre-concentrate (B-MEP) at different ratio surfactant to cosurfactant and with increasing the water content at constant ratio of labrafil and STmix and that is 0.25, in order to find out whether there is any relationship exists between the electro conductivity and viscosity in the microstructure. From the above investigations, six potential microemulsion vehicles (ME7 to ME12) were prepared (Table 5.2) to check the effect of concentration of surfactant and cosurfactant, ratio of surfactant to cosurfactant and concentration of labrafac content on physicochemical properties of microemulsion. At ME7, ME8 and ME9, total % of STmix was constant and that is 60% but surfactant to cosurfactant ratio was varied. In these formulation % of labrafac and water was kept constant at 15% and 25% respectively. Similarly, another three-microemulsion (ME10, ME11 and ME12) formulation was prepared by keeping the concentration of STmix 40% constant but ratio of surfactant to

cosurfactant was varied. Similarly here also % of labrafac and water was constant and that is 10% and 50% respectively. In all the cases oil: STmix (0.25) were kept constant.

Table 5.2: Microemulsions vehicle composition (%w/w) at different ST to COST ratio (Km) (System B)

Batch No	ST to COST ratio	Tween 80	Propylène glycol	Labrafac	Water
ME7	3 :1	45	15	15	25
ME8	2 :1	40	20	15	25
ME9	1 :1	30	30	15	25
ME10	3 :1	30	10	10	50
ME11	2 :1	26.7	13.3	10	50
ME12	1 :1	20	20	10	50

5.2.2.2. Efavirenz:

From the pre-formulation study and phase diagram study, different microemulsion formulation was selected (Table 5.3 and Table 5.4) for further study. Microemulsion was prepared by simple mixing as described earlier. In all the cases the efavirenz was dissolved into the oil and surfactant mixture to get the final concentration of drug in microemulsion was 20mg/ml. All the samples were prepared 48h before investigations for distribution of oil, water and surfactant micelles to attain the thermodynamic equilibrium and were stored at room temperature. These batches were speculated to give reasonably different types of colloidal microstructure, which could be confirmed, by electro-conductivity and viscosity.

5.2.2.2.1. Detailed studies on Labrasol transcutol, labrafil M 1944 CS and water system (System C)

From the constructed pseudo-ternary phase diagram of labrasol/ transcutol/ labrafil M 1944 CS and pre-formulation study it was clear that the maximum solubilization of water was achieved when oil phase corresponds to < 20% irrespective of the ST to COST ratio (Km). Reduction in STmix content or when oil phase contribute > 20% of the oil and STmix showed lesser ability to solubilize the water phase. That's why labrafil M1944

CS: STmix ratio was selected as 0.25 for further investigation. Also we have investigated the structural changes in the microemulsion pre-concentrate (C-MEP) with increasing the water content at constant ratio of labrafil and STmix and that is 0.25, in order to find out whether there is any relationship exists between the electro conductivity and viscosity in the microstructure. From the above investigations, six potential microemulsion vehicles (ME13 to ME18) were prepared (Table 5.3) to check the effect of concentration of surfactant and cosurfactant, ratio of surfactant to cosurfactant and concentration of labrafac content on physicochemical properties of microemulsion. At ME13, ME14 and ME15, total % of STmix was constant and that is 48% but surfactant to cosurfactant ratio was varied. In these formulation % of labrafil M1944CS and water was kept constant at 12% and 40% respectively. Similarly, another three-microemulsion (ME16, ME17 and ME18) formulation was prepared by keeping the concentration of STmix 32% constant but ratio of surfactant to cosurfactant was varied. Similarly here also % of labrafac and water was constant and that is 8% and 60% respectively. In all the cases oil: STmix (0.25) were kept constant.

Table 5.3: Microemulsions vehicle composition (%w/w) at different ST to COST ratio (Km) (System C)

Batch No	ST to COST ratio	Labrasol	Transcutol	Labrafil M 1944CS	Water
ME13	4 :1	38.4	9.6	12	40
ME14	3 :1	36	12	12	40
ME15	2 :1	32	16	12	40
ME16	4 :1	25.6	6.4	8	60
ME17	3 :1	24	8	8	60
ME18	2 :1	21.3	10.7	8	60

5.2.2.2.2. Detailed studies on cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (System D):

From the constructed pseudo-ternary phase diagram of labrasol/ transcutol/ labrafil M 1944 CS and pre-formulation study it was clear that the maximum solubilization of water

was achieved when oil phase corresponds to < 20% irrespective of the ST to COST ratio (Km). Reduction in STmix content or when oil phase contribute > 20% of the oil and STmix showed lesser ability to solubilize the water phase. That's why labrafil M1944 CS: STmix ratio was selected as 0.25 for further investigation. Also we have investigated the structural changes in the microemulsion pre-concentrate (D-MEP) with increasing the water content at constant ratio of labrafil and STmix and that is 0.25, in order to find out whether there is any relationship exists between the electro conductivity and viscosity in the microstructure. From the above investigations, six potential microemulsion vehicles (ME19 to ME24) were prepared (Table5.4) to check the effect of concentration of surfactant and cosurfactant, ratio of surfactant to cosurfactant and concentration of labrafac content on physicochemical properties of microemulsion. At ME19, ME20 and ME21, total % of STmix was constant and that is 56% but surfactant to cosurfactant ratio was varied. In these formulation % of labrafil M1944CS and water was kept constant and that is 14% and 30% respectively. Similarly, another three-microemulsion (ME16, ME17 and ME18) formulation was prepared by keeping the concentration of STmix 40% constant but ratio of surfactant to cosurfactant was varied. Similarly here also % of labrafac and water was constant at 10% and 50% respectively. In all the cases oil: STmix (0.25) were kept constant.

Table 5. 4: Microemulsions vehicle composition (%w/w) at different ST to COST ratio (Km) (System D)

Batch No	ST to COST ratio	Cremophor RH 40	Propylène glycol	Labrafil M 1944CS	Water
ME19	3:1	42	14	14	30
ME20	2:1	37.3	18.7	14	30
ME21	1:1	28	28	14	30
ME22	3:1	30	10	10	50
ME23	2:1	26.7	13.3	10	50
ME24	1:1	20	20	10	50

5.2.3. Drug content in microemulsion:

At 3 days after the preparation of drug loaded microemulsions, the precipitated drug was removed from microemulsions by filtration through a 0.45 mm membrane filter. The amount of drug in the resulting clear filtrate was determined using a UV spectrophotometer after appropriate dilution.

5.2.3.1. Acyclovir:

The concentration of Acyclovir within the microemulsions was analyzed by using an UV spectrophotometer. Briefly, an aliquot of microemulsion sample was taken in 25ml volumetric flask and volume was made up to the mark by with ethanol. The resulted solution was further diluted with ethanol, if necessary, and the absorbance was measured at 252 nm. The concentration of the drug was found from Y= 0.0744 x + 0.053 equation. Triplicate estimations were made and the mean absorbance was determined. Detailed method was described in method development section.

5.2.3.2. Efavirenz:

The concentration of efavirenz within the microemulsions was analyzed by using an UV spectrophotometer. Briefly, an aliquot of microemulsion sample was taken in 25ml volumetric flask and volume was made up to the mark by with methanol. The resulted solution was further diluted with methanol, if necessary, and the absorbance was measured at 247 nm. The concentration of the drug was found from 0.0531x +0.0034 equation. Triplicate estimations were made and the mean absorbance was determined. Detailed method was described in method development section.

5.2.4. Electro conductive measurement and percolation threshold:

The solubilization of water phase in the selected oil mixture was monitored quantitatively by measuring the electrical conductivity (s). The water phase was added drop by drop in the initial mixture of oil and surfactant mixture and measured electrical conductivity of formulated samples, using a conductometer (CM 180 conductivity meter, Elco, India) at ambient temperature. The measurements were made at a constant frequency of 1 Hz. The cell constant was ascertained by using a standard potassium chloride solution. The

adherence effect of the ST and the COST upon the cell inner wall and the electrodes was avoided by pre-washing the cell with distilled water followed by the sample to be measured before each measurement.

5.2.5. Viscosity measurements

Viscosity determination is necessary for microemulsions to characterize the system physically and to control its stability. (Constantinides PP et al 1995) (Ktistis G et al. 1990) Viscosity of the different formulation was evaluated using a Brookfield LVDV 111 + CP viscometer (Mfg: Brookfield, USA) using rheocal software at a temperature of 30 ± 1^0C. Experiments were carried out in triplicate for each sample their viscosity values were obtained by the viscometer at the highest spindle rotation speed (30 rpm).

5.2.6. Dilution effect and particle size measurements

Size analysis of microemulsions was carried out by photon correlation spectroscopy with Zetasizer H_{SA} 3000(Malvern Instruments Ltd., Malvern, UK). A solid-state laser was used as the light source. The photon correlation spectroscopy measurements were carried out at a scattering angle of 90^0 and at 25^0C temperature with polystyrene beads used to calibrate instrument performance. Samples were placed in quartz couvettes. Five different experiments per sample were performed and data are expressed as mean ± standard deviation (S.D.) Microemulsion pre-concentrate (MEP) basic mixture (oil, ST and COST) was diluted with excess (100times) water or 0.1N HCl to simulate gastric fluid dilution and then particle size of the system was also determined.

5.2.7. Zeta potential measurements

A zeta potential for different microemulsion was determined using Zetasizer H_{SA} 3000 (Malvern Instrument Ltd., UK). Samples were injected into the cylindrical couvettes and results were recorded. Before putting the fresh sample couvettes was washed with the methanol and rinsed using the sample to be measured before each experiment. Each sample was analyzed in triplicate

5.2.8. Refractive index and % Transmittance:

Refractive index of the different system was measured by Abbe refractometer by placing one drop of solution in the slide. % Transmittance of the system was measured at 650nm using UV spectrophotometer (UV 1601, Shimadju, Japan) keeping distilled water as blank.

5.2.9. pH Determination:

pH values of acyclovir microemulsions were determined in triplicate at room temperature with a digital pH meter (Elco, India)

5.2.10. In vitro intestinal permeability studies

The methods employed are modified from experimental procedures well described in the literature (Smith et al, 1996). Albino male Sprague–Dawley rats (250–300 g) rats were killed by over-dose with pentobarbitone administered by intravenous injection. To check the intestinal permeability, the upper part of the small intestine was isolated and taken for in vitro diffusion study. Then it was thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. Fixed length of the intestine (2") was taken to carry out the diffusion study. The microemulsion sample was taken and diluted with 1 ml of distilled water and mixed for 30 sec by vortex mixture. The in vitro diffusion study was also carried out against the marketed sample and for pure drug also. The resultant sample was injected inside the lumen of intestine using a syringe and the two side of the intestine was tightly closed. Then it was placed in a chamber of organ bath with continuous aeration and constant temperature at 37^0C. The receiver compartment was filled with 30ml Phosphate buffer saline (pH 7.4) for acyclovir and 1% SLS solution for efavirenz. Teflon coated magnetic bar was placed on the receiver compartment which was stirred at 50rpm. The % diffusion of drug was calculated against time and plotted in the graph.

5.2.10.1. Acyclovir:

Different microemulsion pre-concentrate sample (AMEP and BMEP) of different surfactant and cosurfactant ratio (Km) was taken and diluted by 1 ml of distilled water.

The marketed tablet preparation of acyclovir (OCUVIR- 400, Mfg; FDC, B.No-DCG-4041) was also taken for comparative diffusion study. The tablet and pure acyclovir sample was suspended in distilled water to get the concentration of acyclovir 10mg/ml. Similar to the microemulsion sample, 1 ml of tablet or pure drug suspension was taken and diluted with 1ml of distilled water (outside mixing for 1 minute by vortex mixer). % diffusion of the drug vs. time was calculated using Y= 0.0547 x + 0.043 equation. (R^2= 0.9967)

5.2.10.2. Efavirenz:

Different microemulsion pre-concentrate sample (CMEP and DMEP) of different surfactant to cosurfactant ratio (Km) was taken and diluted by 1 ml of distilled water. The marketed capsule preparation of acyclovir (EFAVIR 200mg, Mfg; CIPLA, B.NO: G467750) was also taken for comparative diffusion study. The content of the capsule was taken and suspended in distilled water to get the concentration of efavirenz 20mg/ml. Similarly suspension of pure efavirenz (20mg/ml) was also made using distilled water. Similar to the microemulsion sample, 1 ml of capsule or pure drug suspension was taken and diluted with 1ml of distilled water (outside mixing for 1 minute by vortex mixer). % diffusion of the drug vs. time was calculated using Y= 0.0436 x +0.0167equation. (R^2= 0.9989)

5.2.11. Data analysis

5.2.11.1. Percent Drug Diffusion

The percentage of drug diffused was determined by the formula

%drug diffused = CrVr / CdVd X 100

Cr = Concentration of drug in the receptor compartment

Vr = Volume of the receptor compartment

Cd = Concentration of drug in the donor compartment

Vd = Volume of the donor compartment

5.2.11.2. Kinetics of release

The order of drug release was determined by plotting graphically percent cumulative drug release versus Time and Percent drug release versus time .

Mean steady state flux

The flux across the cellophane membrane (J) was calculated using the formulae

$J = V (dc / dt)$

Where V = Volume of receptor compartment

dc / dt = Rate of change of concentration with time and was the slope of the drug release versus time curve.

5.2.12. Permeation data analysis

Absorption data was plotted as mean cumulative amount of drug diffused as a function of time. J is flux (steady-state rate of penetration) in $\mu g\ cm^{-2}\ min^{-1}$ and was calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area versus time plot (Ceschel,G.C et al., 2000).

Permeability coefficient of the drug through the membrane (P) was determined using following equation (Zhang, H. et al 1996)

$P = J/Cd$

Where, J is flux calculated at the steady time and Cd is drug concentration on the surface of the mucosa.

5.2.13. Transmission Electron Microscopy (TEM)

To check the particle size and morphology, TEM study was carried out using the HITACHI H- 600 Electron Microscope instruments. Procedure for sample preparation: 0.5 ml sample taken in a completely dry test tube and diluted to 10 ml with distilled water. #00 grid copper mesh placed in petridis and one drop of sample put on that grid with the help of micropipette. Now the grid was placed in the holder and viewed under microscope with an accelerated voltage of 50KV. The size of the particles was monitored

on the screen. Three different photographs taken with the help of the attached camera at three different magnifications

5.2.14. Stability studies

5.2.14.1. Visual Inspection

Shelf life as a function of time and storage temperature was evaluated by visual inspection of the microemulsion system at different time period. Stability was monitored at room temperature for 6 months.

5.2.14.2. Centrifugation

In order to estimate metastable systems, the selected microemulsion vehicles were centrifuged (Remi Laboratories, Mumbai, India) at 10, 000 rpm for 30 minute at 0^0C.

5.2.14.3. Freeze–thaw cycles:

Microemulsions were submitted to a total of three complete cycles, each cycle consisting of 24 h at 25^0C followed by 24 h at – 5 ^{0}C (refrigeration). These cycles were important for determining the ability of the microemulsion to withstand thermal shock, as well as to evaluate chemical stability and physical stability of the disperse system.

5.2.14.4. Temperature effect on particle size of microemulsions:

Microemulsions are subjected to stability studies by storing the sample at different temperature i.e.., at room temperature (25 ± 2^0C) and at 40 ± 2^0C temperatures for a period of six months. . Particle size of the sample was measured using Malvern Particle size analyzer (Zetasizer H_{SA} 3000(Malvern Instruments Ltd., Malvern, UK) at different specific time intervals for a period of six months.

5.3. Results and discussion:

5.3.1. Drug content in microemulsion

5.3.1.1. Acyclovir;

Acyclovir contents of the different microemulsions were determined by UV spectroscopy. After analyzing the samples in triplicate, the percentage of drug content in the microemulsion was shown in Table 5.5. All the microemulsion formulations shows drug content >97%.

Table 5.5: Content of acyclovir in different microemulsion

Batch No	% Drug content (R.S.D)[1]	Batch No	% Drug content (R.S.D)[1]
ME1	99.03 (2.02)	ME7	99.03 (2.92)
ME2	99.56 (2.58)	ME8	98.7 (4.18)
ME3	101.53 (2.49)	ME9	97.36 (1.91)
ME4	97.43 (2.09)	ME10	97.06 (2.0)
ME5	98.16 (3.39)	ME11	98.3 (1.15)
ME6	98.25 (2.35)	ME12	96.54 (2.82)

[1] data are expressed as mean ± relative standard deviation (R.S.D.) and n=3

5.3.1.2. Efavirenz:

Efavirenz contents of the different microemulsions were determined by UV spectroscopy. After analyzing the samples in triplicate, the percentage of drug content in the microemulsion was shown in Table 5.6. All the microemulsion formulations shows drug content >97%.

Table 5.6: Content of Efavirenz in different microemulsion

Batch No	% Drug content (R.S.D)[1]	Batch No	% Drug content (R.S.D)[1]
ME13	99.12 (1.25)	ME19	102.14 (1.26)
ME14	101.24 (1.02)	ME20	98.58 (1.75)
ME15	98.25 (3.35)	ME21	97.85 (2.24)
ME16	99.58 (2.25)	ME22	98.68 (1.89)
ME17	100.25 (1.25)	ME23	99.58 (1.76)
ME18	101.75 (2.24)	ME24	100.25 (2.12)

[1] data are expressed as mean ± relative standard deviation (R.S.D.) and n=3

5.3.2. Electro conductive measurement and percolation threshold:

Electrical conductivity is a structure sensitive property and is frequently used to investigate structural changes in macro and microemulsions. The electrical conductivities in each microemulsion depend on the composition of the continuous phase, as shown in Table 5.7 and Figure 5.1. The conductivities in the lower Surfactant phase and upper surfactant phase are almost independent of water concentration. It can be assumed that the structure of the lower phase and upper-phase are w/o type and o/w type, respectively. For the middle phase in the system, conductivity increase with an increase in concentration of aqueous phase. The structure of the middle phase microemulsion is a bicontinuous structure (L. E. Scriven, K.L et al 1977) (U. Olsson et al, 1986) where as some scientist reported the middle phase microemulsion structure may be a liquid-liquid dispersion model; a mixed dispersion medium consisting of a mixture of oil, water, surfactant and cosurfactant (K. Ogino et al 1989).

5.3.2.1. Acyclovir

5.3.2.1.1. Studies on Labrasol, plurol olique, labrafac and water system (System A):

The electrical conductivity (s) of the selected oily mixture was almost zero as long as the % of aqueous phase was less than 10% (w/w). During the aqueous phase titration up to 45% (w/w), electrical conductivity increases fast for Km=4:1. Similar observation was also made when Km=3:1 or 2: 1. At Km=3:1, electrical conductivity increases fast upto 50% of aqueous phase and at Km=2:1 it was 60%. The conductivity of the system was not affected significantly with further addition of aqueous phase after 45%, 50% and 60% at Km=4:1,3:1 and 2:1 respectively.

With the addition of water, electrical conductivity of these systems slightly increases until the critical value is reached. Then a sudden increase in conductivity is observed with the further addition of water. This phenomenon is known as percolation and the critical fraction of dispersed aqueous phase (? w) at which it occurs is known as percolation threshold (? p) (Bennett et al 1982). In such a system conductivity is governed by a universal law (as mentioned below) independent of the physical properties of the medium. Just before the percolation threshold, the system suddenly become conducting.

$= (? w - ? p)^t$ .....Equation 1

where as s is the electrical conductivity, ? w is the dispersed aqueous volume faction and ? p is the dispersed volume fraction at percolation threshold (M. Lagues et al, 1980) (S. A. Safran et al 1987) and t depends on the system dimensionality (t= 8/5 for 3-dimensional system)

To test the validity of the percolation theory, $s^{1/t}$ was plotted against ? w. Percolation threshold can be find out by extrapolating the straight line which join the middle part of the graph and intersect the ? w axis (Figure 5.3..to Figure 5.5). The increase in microemulsion conductivity observed when the water content is raised above the percolation threshold may result from a progressive aqueous droplet interlinking and clustering process (B. Lagourette et al, 1979). Various mechanisms have been proposed to explain the percolative conduction observed with some w/o microemulsions; one of the most accepted mechanisms iis the model of 'sticky droplet collisions' model suggested by Fletcher and Robinson (P.D.I. Fletcher et al 1981).

The investigated microemulsion system containing Labrasol, an non ionic ST, showed electro conductive behavior in spite of its non-ionic nature. In the lower region of aqueous content w/o microemulsion was formed. Beyond the percolation threshold (? p ~ 10%, 15% and 21% for Km=4:1,3; 1 and 2:1 respectively) conductivity increase linearly and sharply upto 45%, 50% and 60% water content at Km=4:1,3:1 and 2:1 respectively It can be concluded that beyond ? p a network of conductive channels exists which corresponds to the formation of water cylinders or channels in an oil phase due to the attractive interactions between the spherical micro droplets of water phase in the w/o microemulsion (M. Chunsheng et al 2000) (S. Weigert et al 1997). Thus Figure 5.1 represent the structure of microemulsion that is when ? w >10% w/o microemulsion is formed, 45%> ? w >10% shows the bicontinuous structure and when ? w >45% then o/w microemulsions are formed for Km=4:1. Similarly, from Figure 5.1 when Km=3:1, ? w >15% w/o microemulsion is formed, 50%> ? w >15% shows the bicontinuous structure and when ? w >50% then o/w microemulsions are formed. Similarly, from Figure 5.1 when Km=2:1, ? w >20% w/o microemulsion is formed, 60%> ? w >20% shows the bicontinuous structure and when ? w >60% then o/w microemulsions are formed.

5.3.2.1.2. Studies on tween 80, propylene glycol, labrafac and water system (System B):

The electrical conductivity of the selected system was shown that there was gradual increase of conductivity with increasing the aqueous content of the microemulsion system, irrespective of different surfactant to cosurfactant ratio (Km). There was no sudden increase in conductivity observed after gradual addition of aqueous phase. Table 5.7 and Figure 5.2 represent the electro conductive data with different % of aqueous phase. To test the validity of the percolation theory, $s^{1/t}$ was plotted against ? w but it shows a straight-line curve (Figure 5.6).

So it can be concluded that the all the microemulsion in this system was of o/w type, and there was no transition of w/o —— bicontinuous —— o/w system like system A.

Table 5.7: Measurement of electrical conductivity of the selected acyclovir microemulsion formulation at various % of aqueous phase

% aqueous phase	Conductivity (microsiemens)					
	System A			System B		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
0	0.1	0.1	0.1	0	0.1	0.1
5	0.2	0.1	0.1	15.5	26.4	30.2
10	5.4	0.6	0.3	32.8	46.8	52.5
15	13.2	6.54	4.6	56.6	72.5	80.2
20	23.3	18.6	14.9	80	91.2	104.5
25	44.9	37.6	36.5	106.7	116.5	138.9
30	70.4	58.6	54.5	142.7	148.6	168.5
35	102.3	76.2	72.5	178.1	189.3	210.5
40	135.3	93.4	94..5	215.4	231.4	244.6
45	189.5	112.9	116.6	255.4	268.8	284.9

Cont.....

% aqueous phase	Conductivity (microsiemens)					
	System A			System B		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
50	255.4	141.2	146.5	297.2	315.5	322.2
55	ND	193.5	194.5	333.8	354.8	358.2
60	ND	271.1	242.5	364.8	384.6	394.2
65	ND	ND	265.4	406	425.3	428.6
70	ND	ND	280.2	442	482	497.6

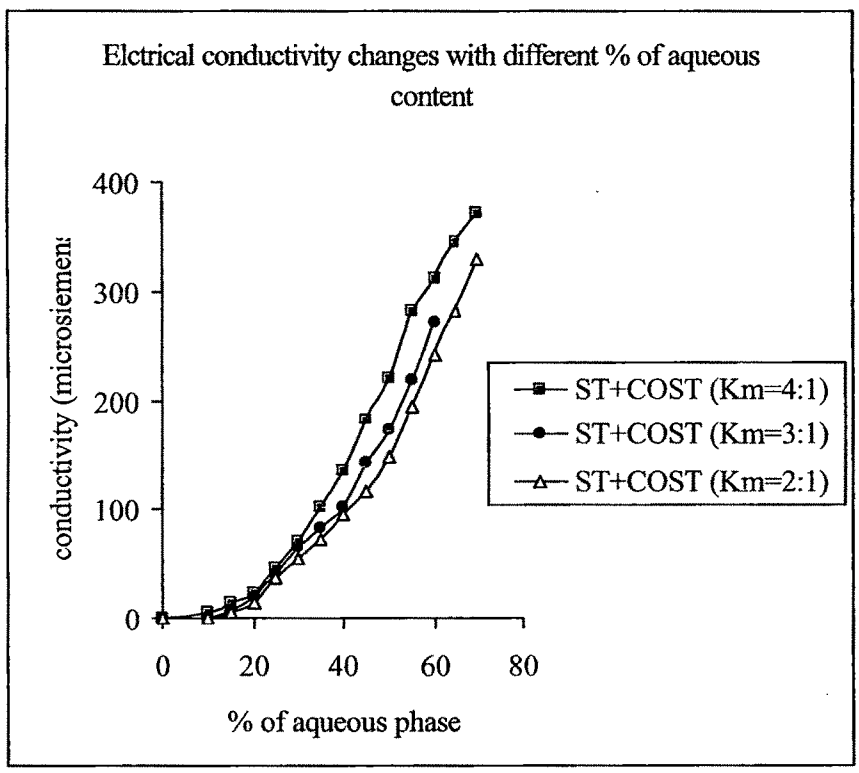

Figure 5.1: Electrical conductivity as a function of water content (System A)

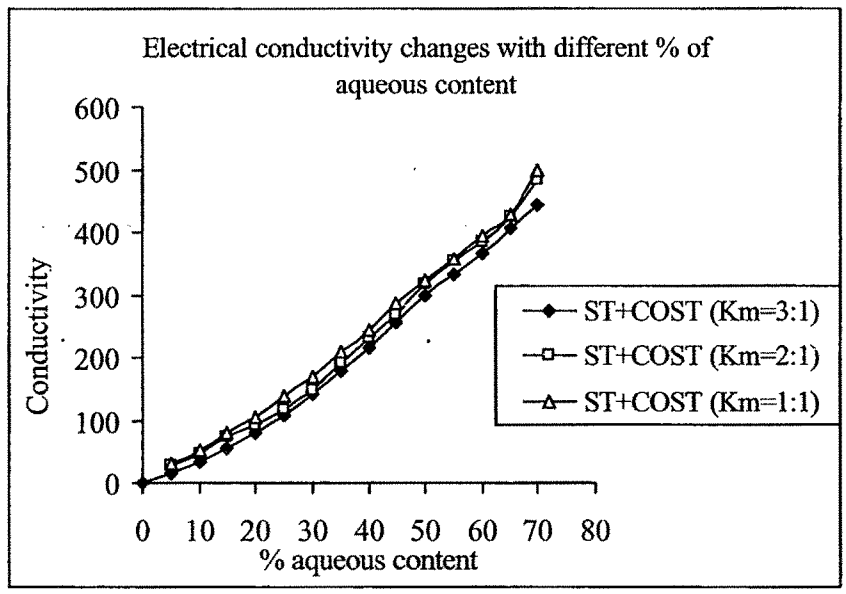

Figure 5.2: Electrical conductivity as a function of water content (System B)

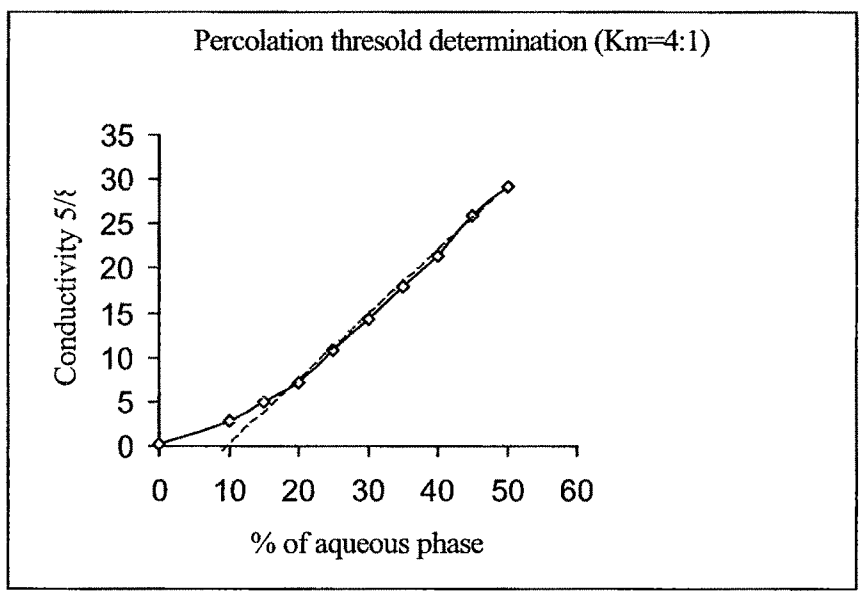

Figure 5.3: Percolation threshold determination of system A (ST/COST ratio=4:1)

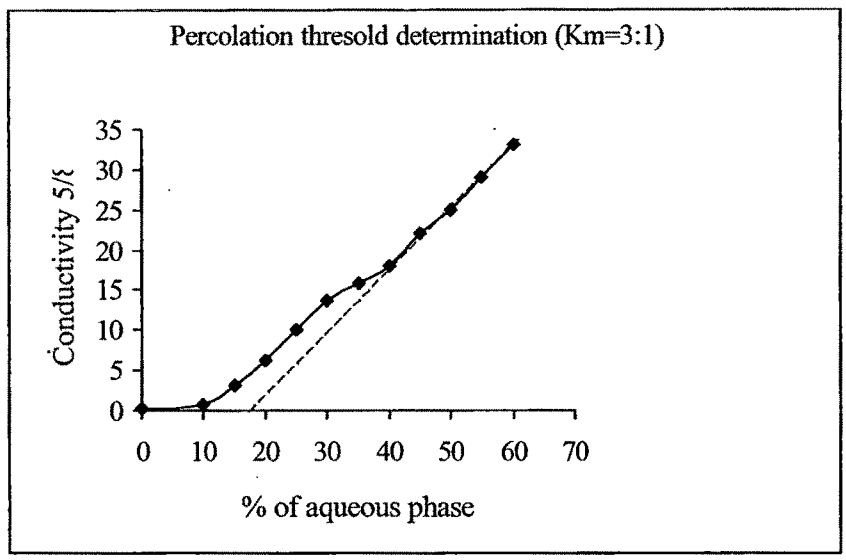

Figure 5.4: Percolation threshold determination of system A (ST/COST ratio=3:1)

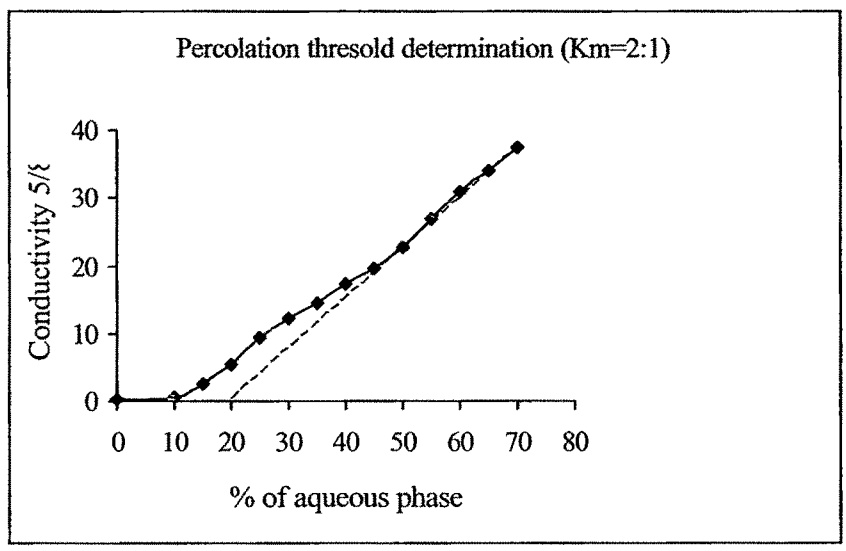

Figure 5.5: Percolation threshold determination of system A (ST/COST ratio=2:1)

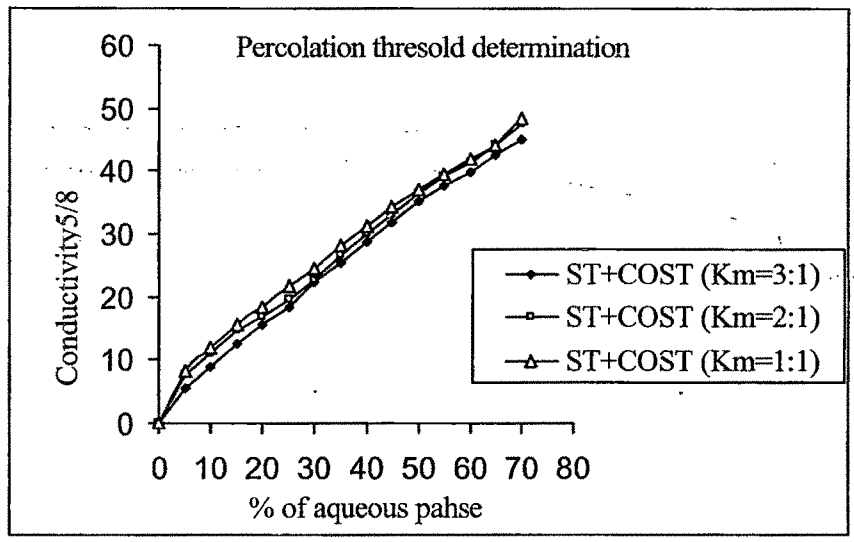

Figure 5.6: Percolation threshold determination of system B at different Km

5.3.2.2. Efavirenz:

5.3.2.2.1. Studies on hbrasol, transcutol, labrafil M 1944 CS and water system (System C)

The electrical conductivity of the selected system was shown that there was gradual increase of conductivity with increasing the aqueous content of the microemulsion system, irrespective of different surfactant to cosurfactant ratio (Km). There was no sudden increase in conductivity was observed after gradual addition of aqueous phase. Table 5.8 and Figure 5.7 represent the electro conductive data with different % of aqueous phase. To test the validity of the percolation theory, $s^{1/t}$ was plotted against ? w but it does not shows any straight line at latter phase (Figure 5.8)

So it can be concluded that the all the microemulsion in this system was of o/w type, and there was no transition of w/o —— bicontinuous —— o/w system like system A.

Table 5.8: Measurement of electrical conductivity of the selected efavirenz microemulsion formulation at various % of aqueous phase

% aqueous phase	Conductivity (microsiemens)					
	System C			System D		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
0	5.26	12.5	15.6	15.8	16.8	19.8
5	21.34	23.5	22.5	19.4	28.6	46.5
10	34.1	48.2	568	36.2	48.9	79.8
15	68.6	85.4	98.5	58.6	72.5	114.5
20	96.3	122.8	142.5	87.3	101.5	152.2
25	126.5	164.5	185.6	114.5	130.2	182.4
30	152.5	194.8	222.5	132.8	154.5	216.8
35	184.8	248.6	265.6	167.9	184.5	252.6
40	512.3	298.8	312.5	192.9	208.9	281.2
45	238.7	341.2	358.6	217.2	232.4	298.6
50	257.4	382.5	412.5	235.8	252.8	312.5
55	279.4	418.2	442.6	257.4	272.5	ND
60	301.4	452.9	489.6	279.4	296.2	ND
65	327.8	498.5	528.6	301.8	319.8	ND
70	353.1	534.8	562.6	320.4	338.5	ND
80	398.4	572.5	589.5	357.4	372.8	ND
90	428.6	612.5	627.8	390.6	412.4	ND

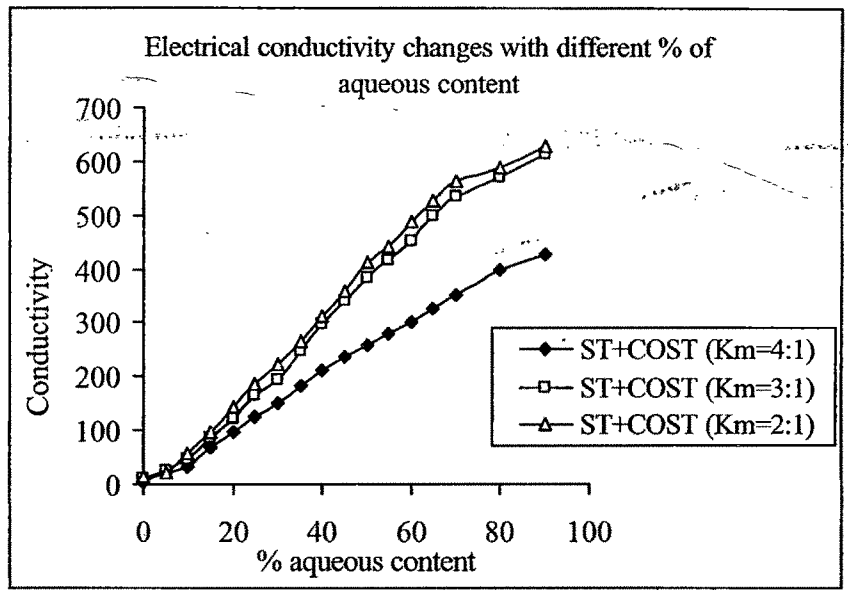

Figure 5.7: Electrical conductivity as a function of water content (System C)

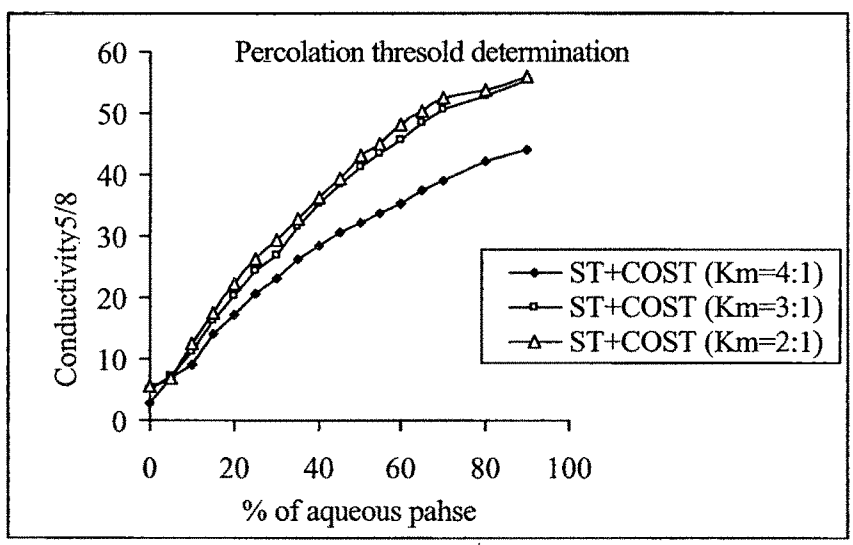

Figure 5.8: Percolation threshold determination of system C at different Km

5.3.2.2.2. Studieson cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (Sytem D)

The electrical conductivity of the selected system was shown that there was gradual increase of conductivity with increasing the aqueous content of the microemulsion system, irrespective of different surfactant to cosurfactant ratio (Km). There was no sudden increase inconductivity was observed after gradual addition of aqueous phase. Table 5.8 and Figure 5.9 represent the electro conductive data with different % of aqueous phase. To test the validity of the percolation theory, $s^{1/t}$ was plotted against ? w but it does notshows any straight line at latter phase (Figure 5.10)

So it can be concluded that the all the microemulsion in this system was of o/w type, and there was no transition of w/o —— bicontinuous —— o/w system like system A.

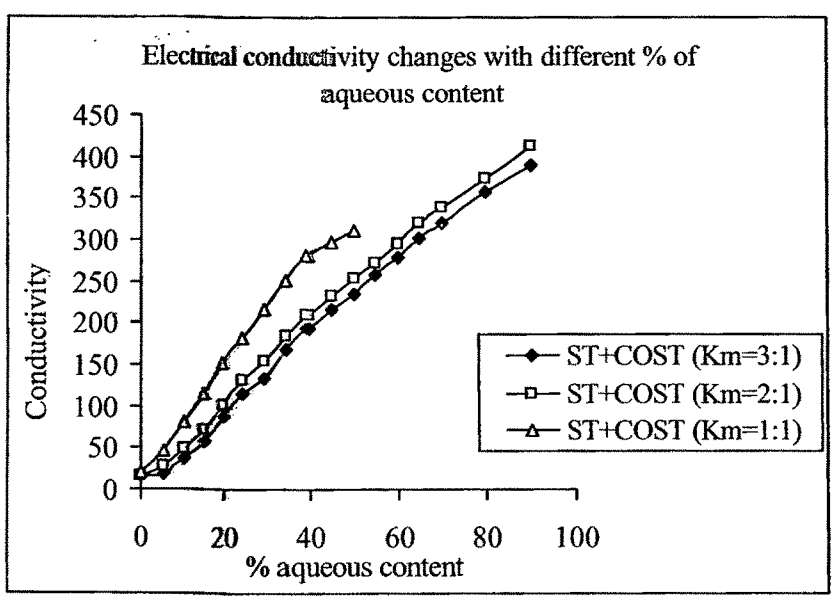

Figure 5.9: Electrical conductivity as a function of water content (System D)

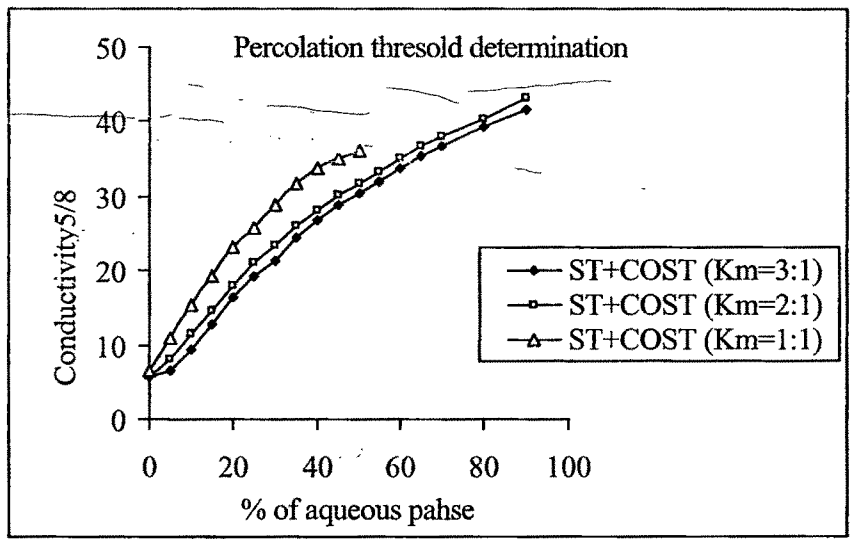

Figure 5.10: Percolation threshold determination of system D at different Km

5.3.3. Viscosity measurements

5.3.3.1. Acyclovir:

The structure of the microemulsion system can be characterized by Rheological measurements. The dependence of the apparent viscosity of the investigated microemulsion was represented in Table 5.9 and Figure 5.11.

Table 5.9: Viscosity (cP) change with increasing % of aqueous phase

% aqueous phase	Viscosity (cP) (R.S.D.)[1]					
	System A			System B		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
0	51.24	61.35	77.38	264.27	247.39	229.82
	(2.43)	(2.46)	(5.24)	(8.65)	(6.58)	(6.97)
10	60.46	68.47	82.42	262.86	237.64	224.57
	(3.25)	(3.52)	(4.78)	(8.21)	(6.42)	(5.64)
15	53.42	70.42	85.52	246.54	215.24	201.28
	(2.43)	(4.68)	(3.54)	(4.95)	(4.62)	(4.68)
20	46.56	60.65	82.42	210.18	142.54	135.24
	(2.62)	(3.64)	(2.46)	(3.52)	(4.58)	(3.46)

Cont.....

% aqueous phase	Viscosity (cP) (R.S.D.) [1]					
	System A			System B		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
30	43.52	49.69	62.58	112.24	46.58	32.27
	(3.82)	(2.82)	(4.62)	(4.12)	(4.68)	(3.46)
40	39.48	44.12	51.99	46.85	18.25	8.66
	(1.94)	(1.95)	(3.51)	(2.65)	(2.74)	(2.58)
50	27.39	34.26	42.13	17.52	9.76	4.97
	(1.82)	(2.46)	(4.65)	(1.96)	(1.75)	(1.68)
60	10.62	16.25	29.39	8.65	5.74	3.65
	(1.21)	(1.46)	(3.24)	(1.12)	(1.28)	(1.46)
70	2.10	4.69	8.45	4.77	3.61	2.92
	(0.68)	(1.12)	(1.46)	(1.10)	(0.98)	(0.94)

[1] data are expressed as mean ± relative standard deviation (R.S.D.) and n=3

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

All the tested samples were of low viscosity (? = 2 – 85 cP). The viscosity of the system increases with increasing water content from 0 to 10% when Km=4:1, from 0 to 15% when Km=3:1 and from 0 to 20% when Km=2:1. After addition of 10% aqueous phase, the viscosity rises from 51.24cp to 60.46cp when Km=4:1, 61.35 to 68.47cp when Km=3:1and from 77.38 cp to 82.42 when Km=2:1. Similarly, the viscosity of the system rises after 15% addition of aqueous phase when Km=3:1 or 2;1 only, but decreases when Km=4:1. The viscosity rises from 68.47 to 70.42 cP for Km=3:1 and 82.42 to 85.52 cP when Km =2:1 after 15% addition of aqueous phase. Further addition of aqueous phase decreases the viscosity of the system irrespective of Km The similar study was reported by Bennett et al that increasing the volume fraction of dispersed phase increases the viscosity and it could be expected that viscosity changes reflects a transformation of system microstructure in the function of dispersed volume fraction. Initial increase of the viscosity with increasing ? w is probably the consequences of attractive interaction and aggregation of droplets of water phase including molecular reorganizations on the

interface. The increase in viscosity may also be due to the formation of bicontinuous structure from droplet-like formation.

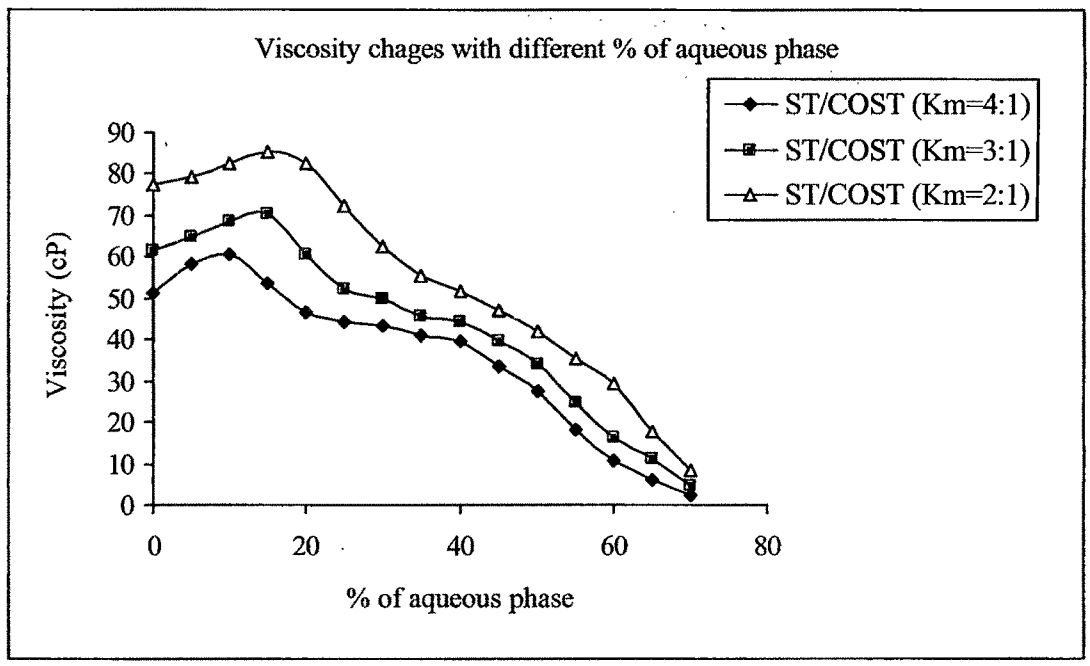

Figure 5.11: Viscosity changes with increasing water content at different surfactant to cosurfactant ratio (Km) (system A)

It was also observed that at Km=4, viscosity of the system gradually and slowly decrease with the increase in the aqueous phase when 10% < ? w<45%, followed by drastic decrease beyond 45%. This is probably due to the fact that the system transforms from w/o (when ? w >10%) through bicontinuous structure (10 %< ? w<45%) to o/w system. The viscosity of the system decrease to 27.39 cP (when ? w = 50%) from 35.62 cP (when (? w =45%).

At Km=3:1, viscosity of the system gradually and slowly decrease with the increase in the aqueous phase when 15% < ? w<50%, followed by drastic decrease beyond 50%. This is probably due to the fact that the system transforms from w/o (when ? w >15%) through bicontinuous structure (15 %< ? w<50%) to w/o system. The viscosity of the system decrease to 16.25 cP (when ? w = 60%) from 34.26 cP (when (? w =50%).

Km=2:1, viscosity of the system gradually and slowly decrease with the increase in the aqueous phase when 20% < ? w<60%, followed by drastic decrease beyond 60%. This is probably due to the fact that the system transforms from w/o (when ? w >20%) through bicontinuous structure (20 %< ? w<60%) to o/w system. The viscosity of the system decrease to 17.32 cP (when ? w = 65%) from 29.39 cP (when (? w =60%). This curve (? – ? w) can also be illustrated by a polynomial equation. Polynomial dependency of viscosity of the system on ? w shows the presence of non-spherical aggregates of dispersed phase. From the viscosity measurements of the investigated system as well as percolation phenomenon, it may be concluded that the system undergoes a structural changes from w/o to o/w over bicontinuous structure as a function of increasing water at fixed temperature. The formation of bicontinuous structure also can be confirmed by electrical conductivity. This is possibly due to the transformation of the interfacial film curvature as concentration of surfactant / cosurfactant is decreased as well as concentration of oil and water phase changes. Based on the conductivity and viscosity results, all the microemulsion systems (ME1-ME12) are o/w type.

5.3.3.1.2. Studies on tween 80, propylene glycol, labrafac and water system (System B)

All the tested samples were of median to ow viscosity (? =2.9 – 264cP). The viscosity of the system decreases with increasing water content, irrespective of the surfactant to cosurfactant ratio (Km). After addition of 10% aqueous phase, the viscosity goes down 264.27cp to 262.86cp when Km=3:1, 247.39 to 237.64cp when Km=2:1and from 229.82cp to 224.57 when Km=2:1. Further addition of aqueous phase sudden decreases the viscosity of the system irrespective of Km(Figure 5.12). After 30% addition of water phase the viscosity goes down to 112.24cp, 46.58 and 32.27cp at Km=3:1, 2:1 and 1:1 respectively.

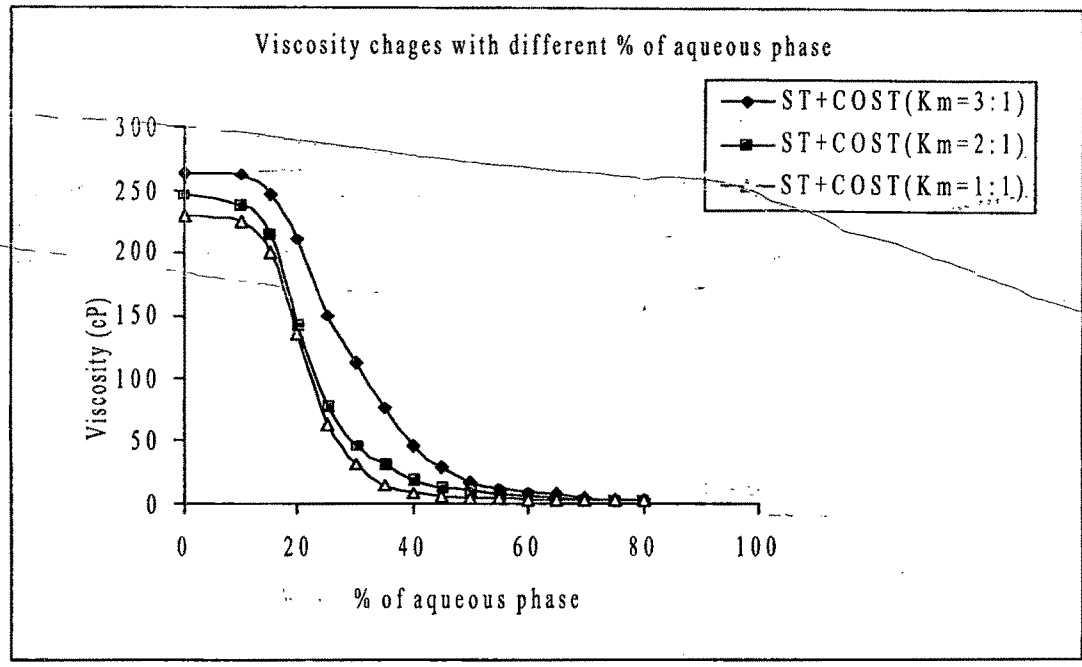

Figure 5.12: Viscosity (cP) change with increasing % of aqueous phase (system B)

5.3.3.2. Efavirenz:

The structure of the microemulsion system can be characterized by Rheological measurements. The dependence of the apparent viscosity of the investigated microemulsion was represented in Table 5.10 and Figure 5.13.

Table 5.10: Viscosity (cP) change with increasing % of aqueous phase

% aqueous phase	Viscosity (cP) (R.S.D.)[1]					
	System C			System D		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
0	44.9	40.4	33.8	318.5	254.2	220.6
	(3.2)	(4.2)	(4.8)	(12.6)	(16.2)	(14.2)
10	42.8	36.4	28.5	327.7	258.2	226.4
	(3.4)	(3.4)	(4.3)	(16.4)	(10.5)	(18.4)
20	33.2	26.4	17.2	228.5	154.4	136.8
	(2.6)	(2.8)	(2.2)	(12.8)	(12.4)	(9.5)
30	19.2	14.4	9.8	99.1	74.6	61.2
	(2.8)	(2.4)	(1.6)	(8.9)	(6.8)	(6.4)

Cont.....

% aqueous phase	Viscosity (cP) (R.S.D.)[1]					
	System C			System D		
	Km=4 :1	Km=3 :1	Km=2 :1	Km=3 :1	Km=2 :1	Km=1 :1
40	10.2	8.5	4.7	42.65	30.4	22.5
	(2.2)	(2.2)	(1.4)	(9.5)	(4.8)	(4.2)
50	4.52	3.4	3.2	20.6	8.6	4.6
	(1.1)	(1.4)	(1.2)	(6.2)	(3.2)	(2.8)
60	3.4	2.7	2.2	4.32	3.6	3.4
	(1.2)	(1.2)	(0.9)	(2.4)	(2.1)	(1.9)
70	2.8	2.4	1.6	3.36	2.8	2.7
	(0.8)	(1.5)	(0.6)	(1.8)	(1.6)	(1.6)
80	2.6	1.9	1.5	2.32	2.2	2.1
	(1.1)	(0.7)	(0.8)	(1.8)	(1.2)	(1.1)

[1] data are expressed as mean ± relative standard deviation (R.S.D.) and n=3

5.3.3.2.1. Studies on labrasol, transcutol, labrafil M 1944 CS and water system (System C)

All the tested samples were of low viscosity (? = 1.5 – 44.9cP). The viscosity of the system decreases with increasing water content, irrespective of the surfactant to cosurfactant ratio (Km). After addition of 10% aqueous phase, the viscosity goes down 44.9cp to 42.8cp when Km=4:1, 40.4 to 36.4cp when Km=3:1and from 33.3 cp to 28.5 when Km=2:1. Further addition of aqueous phase also decreases the viscosity of the system irrespective of Km (Figure 5.13). After gradual addition of water, there was no sudden decrease of viscosity observed like system A. It also proves that there was no transformation of w/o —— bicontinuous ——o/w phase.

5.3.3.2.2. Studies on cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (system D)

All the tested samples were of viscosity ranges from (?) 2.1 to 327.7 cP. The viscosity of the system increases with increasing water content from 0 to 10% irrespective of Km.

After addition of 10% aqueous phase, the viscosity rises from 318.5cp to 327.7cp when Km=3:1, 254.2 to 258.2cp when Km=2:1and from 220.6 cp to 226.4 when Km=1:1. But further addition of aqueous phase decreases the viscosity of the system irrespective of Km (Figure 5.14). The similar study was reported by Bennett et al that increasing the volume fraction of dispersed phase increases the viscosity and it could be expected that viscosity changes reflects a transformation of system microstructure in the function of dispersed volume fraction. Initial increase of the viscosity with increasing water is probably the consequences of attractive interaction and aggregation of droplets of water phase including molecular reorganizations on the interface. The increase in viscosity may also be due to the formation of bicontinuous structure from droplet-like formation.

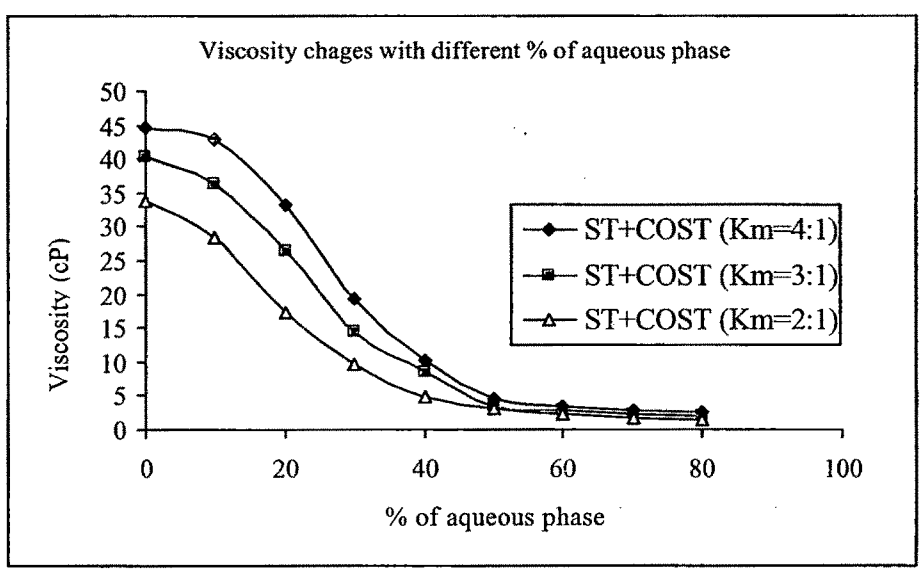

Figure 5.13: Viscosity changes with increasing water content at different surfactant to cosurfactant ratio (Km) (system C)

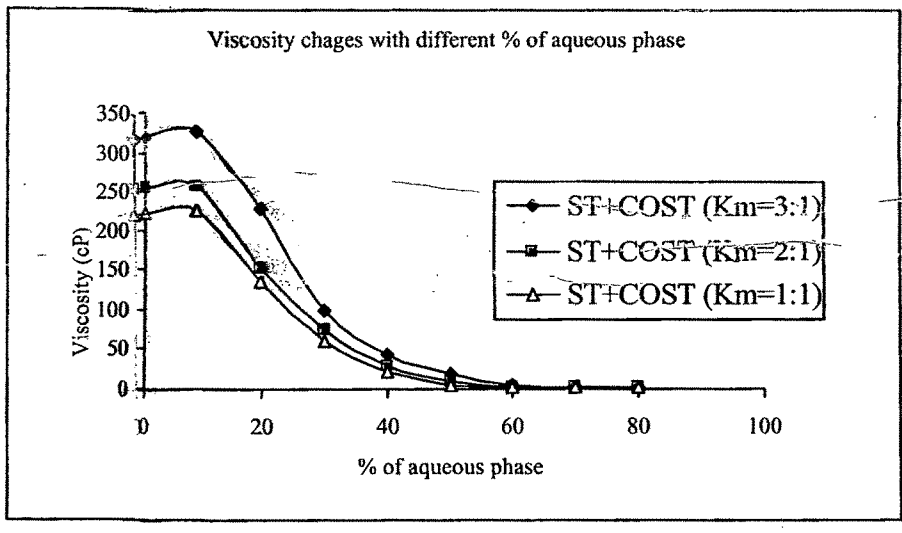

Figure 5.14: Viscosity changes with increasing water content at different surfactant to cosurfactant ratio (Km) (system D)

5.3.4. Dilution effect and particle size measurements

The mean particle size and polydispersity index were calculated from intensity and represented in Table 5.11 and Table 5.12 volume and number bimodal distribution assuming spherical particles. For clarity, however, the results are presented only as intensity distribution. Polydispersity index (PI) is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the polydispersity value the more homogenous are the particles.

5.3.4.1. Acyclovir:

Table 5.11: Particle size (nm) and polydispersity index (PI) data of different microemulsion formulation.

Batch No	Particle size (P.I.)	Batch No	Particle size (P.I.)
ME1	66.4 (0.234)	ME7	85.36 (0.315)
ME2	49.54 (0.412)	ME8	25.36 (0.412)
ME3	23.56 (0.521)	ME9	14.62 (0.225)
ME4	26.94 (0.348)	ME10	16.89 (0.372)
ME5	19.54 (0.421)	ME11	13.25 (0.214)
ME6	20.45 (0.268)	ME12	5.26 (0.125)

Table 5.12: Effect of dilution on particle size (nm) and polydispersity index (P.I.)

B. No	Km	Particle size (P.I.)		B. No	Km	Particle size (P.I.)	
		x 100 w[1]	X 100 SGF[2]			x 100 w[1]	x 100 SGF[2]
A-MEP	4:1	552.42 (0.552)	642.35 (0.616)	B-MEP	3:1	64.25 (0.212)	69.85 (0.248)
A-MEP	3:1	458.65 (0.628)	463.58 (0.612)	B-MEP	2:1	85.26 (0.138)	94.76 (0.172)
A-MEP	2.1	486.52 (0.554)	312.25 (0.558)	B-MEP	1.1	29.38 (0.185)	35.12 (0.196)

*MEP represents for microemulsion pre-concentrate where no water phase was present. It was the mixture of oil and STmix. A- MEP represents microemulsion pre-concentrate for labrasol, plurol olique, labrafac and water system (system A) at labrafac : STmix ratio = 0.25. B- MEP represents microemulsion pre-concentrate for tween 80, propylene glycol, labrafac and water system (system B) at labrafac : STmix ratio = 0.25.

[1] MEP diluted 100 times with distilled water.

[2] MEP diluted 100 times with simulated gastric fluid (0.1N HCl)

5.3.4.1.1. Studies on labrasol, plurol olique, labrafac and water system (System A):

From the Table 5.11, it may be concluded that, all the formulation shown very small particle size range. It was shown from the table that increase % of plurol olique reduces the particle size at fixed concentration of labrafac (15%) and water (25%). As shown in Table 5.11, in ME1 where % of plurol olique was 12%, average particle size was 66.4nm but on increasing the % of plurol olique (reducing the Km from 4:1 to 3:1 or 2:1) shows smaller particle size e.g., 49.54nm and 23.56nm when % of plurol olique was 15% and 20% respectively. Similar observation also noted when % labrafac was 12% and % of water was 40%.

By increase the plurol olique quaintly decreases the particle size. In ME4 where % of plurol olique was 9.6%, shows an average particle size 26.94nm but on ME5 where % of plurol olique was 12% shows a particle size of 19.54nm. But further increase of plurol olique does not reduce the particle size as in ME6 (% of plurol olique was 16%)
Average particle size was 20.45nm.

It was also observed from the Tables 5.11 & Table 5.1 that, at fixed ratio of surfactant to cosurfactant (Km), addition of water from 25% to 40% decreases the particle size when quantity of all other components remains unchanged. As shown in ME1 (Km=4:1), the particle size was 66.4nm when % of water 25% but further dilution of the system with water (upto 40%) decreases the particle size as in ME4 (Km=4:1) where average particle size was 26.94nm. Similar observation also made for other ratio of labrasol to plurol olique also. When Km=3:1, the average particle size decreases to 19.54 nm (ME5) from 49.54nm (ME2) on addition of water from 25% to 40%. Similarly for Km=2:1, the average particle size decreases to 20.45nm (ME6) from 23.56nm (ME3).

It was also observed from the Table 5.11 that, polydispersity index ranges from 0.268 to 0.521, which suggest that the distributions of particles are not completely homogenous.

It was observed from the Table 5.12 that upon 100fold dilution with water, microemulsion preconcentrate (A-MEP) shows a milky white emulsion with average particle size ~ 552nm (Km=4:1), ~458nm (Km=3:1), ~ 486nm (Km=2:1). Polydispersity index (PI) of the system also increases upon 100 times dilution with water. It goes up to 0.552, 0.628 and 0.554 for Km=4:1, 3:1 and 2:1 respectively. High PI suggests the

formation of heterogeneous coarse o/w microemulsion and / or multiple emulsions (w/o/w) (P.P. Constantinides and Seahg H et al 1995).

Microemulsion pre-concentrate (AMEP) at different Km 4:1, 3:1 and 2:1, upon 100 fold dilution with 0.1N HCl shows similar behavior as with water and form milky white emulsion with a particle size of ~ 642nm., 463nm and 312nm. This may be due to the non-ionic component (surfactant and oil) of the microemulsion system, which is insensitive to pH and /or ionic strength changes during dilution. This suggests that system suitability even when it is diluted by body fluid like 0.1N HCl (simulated gastric fluid). This provided useful background on the efficiency of these systems as drug carriers that allow infinite microemulsion dilution when the systems are diluted with body fluids.

5.3.4.1.2. Studies on tween 80, propylene glycol, labrafac and water system (System B):

It was observed from the table that, all the microemulsion formulation having very small particle size. It was shown from the Table 5.11 that increase % of propylene glycol reduces the particle size when concentration of labrafac (15%) and water (25%) was fixed. As shown in Table 5.11, in ME7 where % of propylene glycol was 15%, average particle size was 85.36 nm but on increasing the % of propylene glycol (reducing the Km from 3:1 to 2:1 or 1:1) shows smaller particle size e.g., 25.36nm and 14.62nm when % of propylene glycol was 20% and 30% respectively.

Similar observation also noted when labrafac was 10% and water was 50%. Increasing the propylene glycol quaintly decreases the particle size. ME10 where % of propylene glycol was 10%, showed an average particle size 16.89nm while ME11 with 13.3 % propylene glycol showed a particle size of 13.25nm. Further increases of propylene glycol also reduce the particle size as in ME12 (% of propylene glycol was 20%) where average particle size was 5.26nm.

It was also observed from the Tables 5.11 & Table 5.2 that, at fixed ratio of surfactant to cosurfactant (Km), addition of water from 25% to 50% decreases the particle size when quantity of all other components remains unchanged. As shown in ME7 (Km=3:1), the particle size was 85.36nm when water was 25% but further dilution of the system with water (upto 50%) decreases the particle size (16.89nm) as in ME10 (Km=2:1). Similar

observation also made for other ratio of tween 80 to propylene glycol. When Km=2:1, the average particle size decreases to 13.25 nm (ME11) from 25.36nm (ME8) on addition of water from 25% to 50%. Similarly for Km=1:1, the average particle size decreases to 5.26nm (ME12) from 14.62nm (ME9).

It was also observed from the Table 5.11 that, polydispersity index ranges from 0.125 to 0.412, which suggest that the distribution of particles are not completely homogenous but better in compare to system A.

It was observed from the Table 5.12 that upon 100fold dilution with water, microemulsion pre-concentrate (B- MEP) shows a transparent émulsion with average particle size ~64nm (Km=3: 1), ~85nm (Km=2:1), ~ 29nm (Km=1:1). Polydispersity index (PI) of the system decreases upon 100 times dilution with water. It changes to 0.212, 0.138 and 0.185 for Km=3:1, 2:1 and 1:1 respectively. Low PI suggests the formation of homogenous o/w microemulsion (P.P. Constantindes and Seahg H et al 1995).

Microemulsion pre-concentrate (BMEP) at different Km 3:1, 2:1 and 1:1, upon 100 fold dilution with 0.1N HCl shows similar behavior as with water and form transparent emulsion with a particle size of ~ 69nm, ~94nm and ~35nm. This may be due to the non-ionic component (surfactant and oil) of the microemulsion system, which is insensitive to pH and /or ionic strength changes during dilution. This suggests that system suitability even when it is diluted by body fluid like 0.1N HCl (simulated gastric fluid). This provided useful background on the efficiency of these systems as drug carriers that allow infinite microemulsion dilution when the systems are diluted with body fluids.

5.3.4.2 Efavirenz:

Table 5.13: Particle size (nm) and polydispersity index (PI) data of different microemulsion formulation.

Batch No	Particle size (P.I.)	Batch No	Particle size (P.I.)
ME13	15.2 (0.12)	ME19	19.6 (0.22)
ME14	11.4 (0.14)	ME20	38.5 (0.23)
ME15	16.2 (0.21)	ME21	21.8 (0.18)
ME16	15.6 (0.14)	ME22	16.5 (0.09)
ME17	6.8 (0.12)	ME23	22.4 (0.14)
ME18	8.7 (0.19)	ME24	14.6 (0.21)

Table 5.14: Effect of dilution on particle size (nm) and polydispersity index (P.I.)

B. No	Km	Particle size (P.I.)		B. No	Km	Particle size (P.I.)	
		x 100 w[1]	X 100 SGF[2]			x 100 w[1]	x 100 SGF[2]
C-MEP	4:1	342.6 (0.525)	364.8 (0.621)	D-MEP	3:1	22.4 (0.11)	25.5 (0.16)
C-MEP	3:1	389.8 (0.415)	414.4 (0.482)	D-MEP	2:1	53.8 (0.17)	44.8 (0.32)
C-MEP	2.1	425.6 (0.395)	394.8 (0.412)	D-MEP	1.1	42.8 (0.24)	31.2 (0.21)

*MEP represents for microemulsion pre-concentrate where no water phase was present. It was thé mixture of oïl and STmix. C- MEP represents microemulsion pre-concentrate for labrasol, transcutol, labrafil M1944 CS (system C) at labrafil M 1944CS : STmix ratio = 0.25. D- MEP presents microemulsion pre-concentrate for cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (system D) at labrafil M 1944 CS : STmix ratio = 0.25.

[1] MEP diluted 100 times with distilled water.

[2] MEP diluted 100 times with simulated gastric fluid (0.1N HCl)

5.3.4.2.1. Studies on llabrasol, transcutol, labrafil M1944CS and water system (System C) :

From the Table 5.13, it may be concluded that, all the formulation shown very small particle size range. It was shown from the table that increase % of transcutol reduced the particle size at fixed concentration of labrafil M 1944CS (12%) and water (40%). ME13 where % of transcutol was 9.6%, average particle size observed was 15.2nm while on increasing the % of transcutol (12%) (reducing the Km from 4:1 to 3:1) showed smaller particle size e.g., 11.4nm when transcutol was 12%. But further increase of transcutol did not decrease the particle size as shown in ME15 where particle size average was 16.2nm and transcutol was 16%.

Similar observation also noted when labrafil M 1944 CS was 8% and water was 60%.

By increase the transcutol quaintly decreases the particle size. In ME16 where transcutol was 6.4%, shows an average particle size 15.6nm but on ME16 where transcutol was 8% shows a particle size of 8nm. But further increase of transcutol does not reduce the particle size as in ME18 (transcutol was 10.7%) and average particle size was 8.7nm.

It was also observed from the Table 5.13 & Table 5.3 that, at fixed ratio of surfactant to cosurfactant (Km), addition of water from 40% to 60% decreases or unchanged the particle size when quantity of all other components remains unchanged. As shown in ME13 (Km=4:1), the particle size was 15.2nm when water was 40% but further dilution of the system with water (upto 60%) does not affect the particle size as in ME16 (Km=4:1) where average particle size was 15.6nm. But when Km=3:1, the average particle size decreases to 6.8 nm (ME17) from 11.4nm (ME13) on addition of water from 40% to 60%. Similarly for Km=2:1, the average particle size decreases to 8.7nm (ME18) from 16.2nm (ME15).

It was also observed from the Table 5.13 that, polydispersity index ranges from 0.12 to 0.21, which suggest that the distributions of particles are homogenous. It was observed from the Table 5.14 that upon 100fold dilution with water, microemulsion pre-concentrate (C- MEP) shows a milky white emulsion with average particle size ~342nm (Km=4: 1), ~389nm (Km=3:1), ~ 425nm (Km=2:1). Polydispersity index (PI) of the system also increases upon 100 times dilution with water. It goes up to 0.525, 0.415 and 0.395 for Km=4:1, 3:1 and 2:1 respectively. High PI suggests the formation of

heterogeneous coarse o/w microemulsion and / or multiple emulsions (w/o/w) (P.P. Constantindes and Seahg H et al 1995).

Microemulsion pre-concentrate (CMEP) at different Km 4:1, 3:1 and 2:1, upon 100 fold dilution with 0.1N HCl shows similar behavior as with water and form milky white emulsion with a particle size of ~ 364nm., ~ 414nm and ~ 394nm. This may be due to the non-ionic component (surfactant and oil) of the microemulsion system, which is insensitive to pH and /or ionic strength changes during dilution. This suggests that system suitability even when it is diluted by body fluid like 0.1N HCl (simulated gastric fluid). This provided useful background on the efficiency of these systems as drug carriers that allow infinite microemulsion dilution when the systems are diluted with body fluids.

5.3.4.2.2. Studies on Cremophor RH 40, propylene glycol, labrafil M 1944CS and water system (system D) :

It was observed from the Table 5.13 that, all the microemulsion formulation having very small particle size. It was shown from the Table 5.13 that there was no direct correlation with % of propylene glycol and particle size. At fixed concentration of labrafil M 1944 CS (14%) and water (30%), the increase propylene glycol increases the particle size when ratio of Km changes from 3:1 to 2:1 but decreases when ratio of Km changes from 2:1 to 1:1 As shown in Table 5.13, in ME19 where propylene glycol was 14%, average particle size was 19.6nm but on increasing the propylene glycol (reducing the Km from 3:1 to 2:1) shows larger particle size e.g., 38.5nm but further increase of propylene glycol (reducing Km from 2:1 to 1:1) reduces the particle size to 21.8nm when propylene glycol was 18.7% and 28% respectively.

Similar observation also noted when labrafil M 1944 CS was 10% and water was 50%. Increase the propylene glycol quaintly increases or decreases the particle size. In ME22 where % of propylene glycol was 10%, shows an average particle size 16.5nm but on ME23 where % of propylene glycol was 13.3% shows a particle size of 22.4nm. But further increases of propylene glycol reduce the particle size as in ME24 (% of propylene glycol was 20%) where average particle size was14.6nm.

It was also observed from the Table 5.13 & Table 5.14 that, at fixed ratio of surfactant to cosurfactant (Km), addition of water from 30% to 50% decreases the particle size when

quantity of all other components remains unchanged. As shown in ME19 (Km=3:1), the particle size was 19.6nm when water was 40% but further dilution of the system with water (upto 50%) decreases the particle size as in ME22 (Km=2:1) where average particle size was 16.5nm. Similar observation also made for other ratio of cremophor RH40 to propylene glycol. When Km=2:1, the average particle size decreases to 22.4nm (ME23) from38.5nm (ME20) on addition of water from 25% to 50%. Similarly for Km=1:1, the average particle size decreases to 14.6nm (ME24) from 21.8nm (ME21).

It was also observed from the Table 5.13 that, polydispersity index ranges from 0.09 to 0.23, which suggest that the distribution of particles are not completely homogenous but better in compares to system A, B or C.

It was observed from the Table 5.14 that upon 100 fold dilution with water, microemulsion pre-concentrate (D- MEP) shows a transparent emulsion with average particle size ~22nm (Km=3:1), ~53nm (Km=2:1), ~ 42nm (Km=1:1). Polydispersity index (PI) of the system changes upon 100 times dilution with water. It changes to 0.11, 0.17 and 0.24 for Km=3:1, 2:1 and 1:1 respectively. Low PI suggests the formation of homogenous o/w microemulsion (P.P. Constantindes and Seahg H et al 1995).

Microemulsion pre-concentrate (D-MEP) at different Km 3:1, 2:1 and 1:1, upon 100 fold dilution with 0.1N HCl shows similar behavior as with water and form transparent emulsion with a particle size of~ 25nm, ~44nm and ~31nm. This may be due to the non-ionic component (surfactant and oil) of the microemulsion system, which is insensitive to pH and /or ionic strength changes during dilution. This suggests that system suitability even when it is diluted by body fluid like 0.1N HCl (simulated gastric fluid). This provided useful background on the efficiency of these systems as drug carriers that allow infinite microemulsion dilution when the systems are diluted with body fluids.

5.3.5. Zeta potential measurements

Surfactants can stabilize the emulsion, not only just by forming a mechanical barrier, but also by producing an electrical (electrostatic) barrier or surface charge. The electrical surface charge of the droplets is produced by the ionization of interfacial film-forming components. The surface potential and the resulting zeta-potential of emulsion droplets will depend on the extent of ionization of surfactants. (S. Benita et al 1986)

Table 5.15: Zeta potential of the different microemulsion system.

B. No	Zeta (mV)	B. No	Zeta (mV)	B. No	Zeta (mV)	B. No	Zeta (mV)
ME01	18.6	ME07	3.4	ME13	11.2	ME19	20.4
ME02	19.9	ME08	3.8	ME14	10.6	ME20	21.6
ME03	18.2	ME09	4.1	ME15	9.82	ME21	18.4
ME04	14.4	ME10	2.9	ME16	11.5	ME22	19.8
ME05	12.9	ME11	3.9	ME17	12.8	ME23	22.5
ME06	15.4	ME12	4.2	ME18	9.58	ME24	21.2

All the formulations studied were composed of constituents of positive surface charge (Table 5.15). The microemulsion formulation although consist of non-ionic component, showed high positive surface charge which suggest the possibility of better adherence to the intestinal mucosa which is negatively charged. This may also increase the bioavailability of poorly absorbable drugs incorporated in it, by increasing the residence time into the stomach as well as in intestine. (T. Gershanik et al 1998)

5.3.6. Refractive index and % Transmittance:

Transparency of the system was checked and confirmed by measuring refractive index (RI) as well as % transmittance (%T). At first RI of the distilled water was measured to find out the suitability of the instrument and it was found 1.333, which was similarly with the reported data. Table 5.16 shows the RI of the system.

Similarly, %transmittance >95% confirms the transparency of the system. Table 5.16 shows the %T of all the system and for all the cases it shows %T>95%.

Table 5.16: Refractive index (RI) and % Transmittance (%T) of the microemulsion system

B. No	**RI**	**%T**	**B. No**	**RI**	**%T**	**B. No**	**RI**	**%T**	**B. No**	**RI**	**%T**
ME1	1.436	96.2	ME7	1.398	98.6	ME13	1.426	98.7	ME19	1.435	95.6
ME2	1.426	98.2	ME8	1.428	97.6	ME14	1.362	98.3	ME20	1.428	97.8
ME3	1.462	97.6	ME9	1.462	98.2	ME15	1.472	96.8	ME21	1.438	98.4
ME4	1.392	98.6	ME10	1.376	97.6	ME16	1.396	98.6	ME22	1.385	95.4
ME5	1.493	96.8	ME11	1.425	98.4	ME17	1.462	99.2	ME23	1.395	96.2
ME6	1.432	97.2	ME12	1.398	97.2	ME18	1.418	96.6	ME24	1.376	97.8

5.3.7. pH Determination:

Table shows the different pH of the microemulsion formulation. From the Table 5.17 it was observed that all the microemulsion sample within pH range of 6-8.

Table 5.17: pH of the different microemulsion

B. No	pH (S.D.)[1]	B. No	pH (S.D.)[1]	B. No	pH (S.D.)[1]	B. No	pH (S.D.)[1]
ME1	6.46 (0.12)	ME7	6.95 (0.21)	ME13	7.44 (0.16)	ME19	7.54 (0.22)
ME2	6.82 (0.21)	ME8	6.84 (0.13)	ME14	7.61 (0.21)	ME20	7.42 (0.15)
ME3	7.29(0.32)	ME9	6.91 (0.23)	ME15	7.68 (0.14)	ME21	7.85 (0.16)
ME4	6.63 (0.22)	ME10	7.08 (0.18)	ME16	7.57 (0.120	ME22	7.64 (0.13)
ME5	6.92 (0.13)	ME11	6.92 (0.15)	ME17	7.64 (0.21)	ME23	7.84 (0.21)
ME6	7.46 (0.14)	ME12	6.96 (0.24)	ME18	7.75 (0.12)	ME24	7.58 (0.13)

[1] data are expressed as mean ± standard deviation (S.D.) and n=3

5.3.8. In vitro intestinal permeability studies

5.3.8.1. Acyclovir:

In vitro intestinal permeability data was shown in Figure 5.15 to Figure 5.18. It was observed that drug was diffused at faster rate form the microemulsion system as compared to the tablet dosage form. The total percentage diffusion was much higher in case of microemulsion system as compared to the tablet dosage form. After 5 hour of diffusion 96% drug was diffused from the microemulsion system as compared to 69% diffused form tablets.

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

Figure 5.15 shows the in vitro diffusion study of acyclovir through rat intestine from system A as well as from a commercial tablet and pure drug suspension. From the above *in-vitro* diffusion study it was observed that all the microemulsion formulation shows greater absorption as compared to the pure drug and from tablet formulation through rat intestine. It was also observed that maximum absorption takes place when surfactant to cosurfactant ration (Km) was 4:1 as compared to when Km=2:1 or 3:1 for system A.

After 5 hr of diffusion study only 22.1% of drugs got diffused from pure drug suspension. At similar time 66.5% 96.0, 84.6%, and 80.2% of acyclovir got diffused from tablet, Km=4:1, Km=3:1 and Km=2:1 respectively.

It was evident from the above data that maximum absorption takes place, in case of microemulsion, when surfactant to cosurfactant ratio (Km) was 4:1 as compared to Km=3:1 or 2:1. For this reason, *in-vivo* study was carried out using Km=4:1 only.

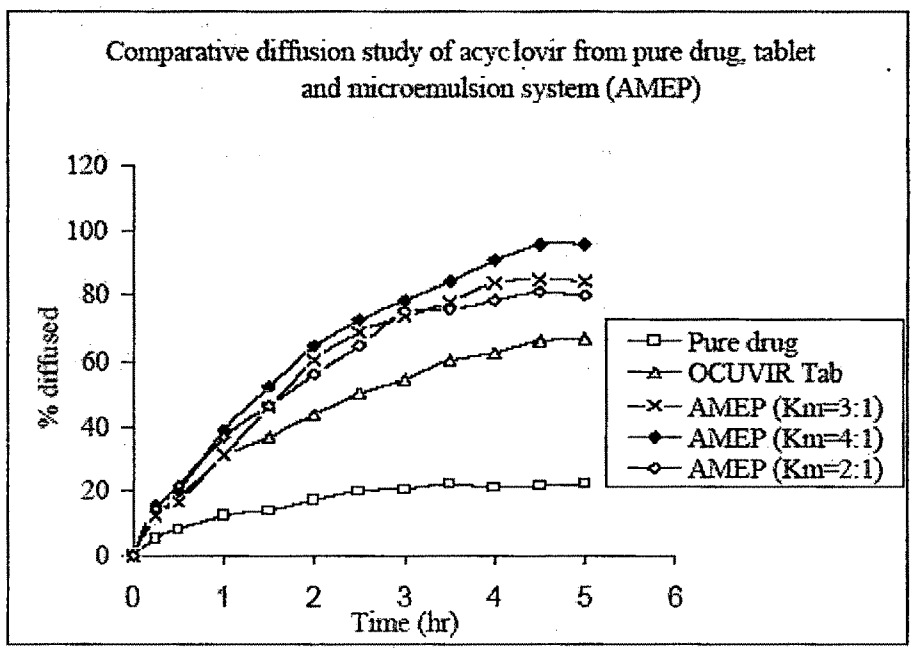

Figure 5.15: Comparative *in-vitro* diffusion profile of acyclovir through rat intestine from pure drug, tablet and microemulsion (AMEP)

The concept of in vitro permeation studies to calculate the quantity of drug remaining in the rat skin has been reported earlier by our group (Rao,Gita et al 2000). The same concept has been extended to analyze the quantity of the drug diffused though rat intestine. Table 5.18 summarizes the mean flux rate (J), permeation constant (P) and diffusion coefficient (D) of pure drug, tablet and microemulsion formulation. Formulations exhibited higher flux value (J), higher permeation coefficient (P) as well as higher diffusion coefficient (D) across the membrane compared to the other formulation, which may be due to either higher release rate from the formulation or higher retention of the drug inside the membrane resulting in lower concentration in the receptor

compartment following absorption. Formulation AMEP (4:1) shows higher flux rate and higher permeation constant compare to the other formulation.

Table 5.18: Comparative diffusion parameter of acyclovir from pure drug, tablet and system A

Formulation	J($\mu g\ cm^{-2}\ min^{-1}$)	P ($cm\ min^{-1}$)	D ($cm^{-2}\ min^{-1}$)
Pure drug	24.771	0.006193	7.92 E-07
Ocuvir tab	66.375	0.016594	2.18 E-06
AMEP (4:1)	**94.33**	**0.023608**	**1.51 E-05**
AMEP (3:1)	86.129	0.021532	1.17 E-05
AMEP (2:1)	84.464	0.021116	1.05 E-05

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

Figure 5.16 shows the in vitro diffusion study of acyclovir from system B through rat intestine. From the above *in-vitro* diffusion study it was observed all the microemulsion formulation shows greater absorption as compared to the pure drug and tablet formulation through rat intestine (Figure 5.15 and Figure 5.16). It was also observed that maximum absorption takes place when Km =2:1 as compared to when Km=3:1 or 1:1 for system A. From Figure 5.16 it was observed that, after 5hr of diffusion study only 22.1% and 66.5% of drugs get diffused from pure drug suspension and tablet suspension respectively but from Figure 5.16 it was observed that at similar time, 98.2%, 97.8% and 86.2% of acyclovir get diffused when surfactant to cosurfactant ratio (Km) was 3:1, 2:1 and 1:1 respectively.

It was also observed that similar and greater absorption from microemulsion when surfactant to cosurfactant ratio (Km) was 3:1 or 2:1 as compared to Km = 1:1. For this reason, *in-vivo* study was carried out using when Km=2:1 only, considering less quantity of surfactants.

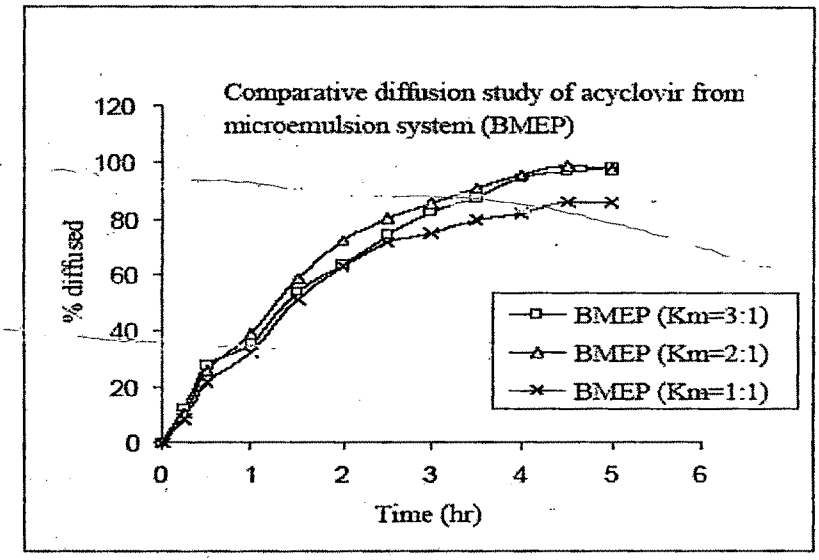

Figure 5.16: *In-vitro* diffusion profile of acyclovir through rat intestine from microemulsion (BMEP)

Table 5.19 summarizes the mean flux rate (J), permeation constant (P) and diffusion coefficient (D) of pure drug, tablet and microemulsion formulation. Formulations exhibited higher flux value (J), higher permeation coefficient (P) as well as higher diffusion coefficient (D) across the membrane compared to the other formulation, which may be due to either higher release rate from the formulation or higher retention of the drug inside the membrane resulting in lower concentration in the receptor compartment following absorption. Formulation BMEP (2:1) shows higher flux rate and higher permeation constant compare to the other formulation.

Table 5.19: Comparative diffusion parameter of acyclovir from pure drug, tablet and system B

Formulation	J($\mu g\ cm^{-2}\ min^{-1}$)	P ($cm\ min^{-1}$)	D ($cm^{-2}\ min^{-1}$)
Pure drug	24.771	0.006193	7.92 E-07
Ocuvir tab	66.375	0.016594	2.18 E-06
BMEP (3:1)	97.201	0.0243	1.58 E-05
BMEP (2:1)	**102.03**	**0.025508**	**1.56E-05**
BMEP (1:1)	89.02	0.022255	1.22E-05

5.3.8.2. Efavirenz:

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

Figure 5.17 shows the in vitro diffusion study of efavirenz through rat intestine from system C as well as from capsule and pure drug suspension. From the above *in-vitro* diffusion study it was observed all the microemulsion formulation shows greater absorption as compared to the pure drug and capsule formulation through rat intestine. It was also observed that maximum absorption takes place when surfactant to cosurfactant ratio (Km) was 4:1 as compared to when Km=3:1 or 2:1 for system C. After 5 hr of diffusion study only 8.2% of drugs get diffused from pure drug suspension. At similar time, 54.2% 95.6%, 89.8% and 83.4% of efavirenz get diffused from capsule, CMEP (Km=4:1), CMEP (Km=3:1) and CMEP (Km=2:1) respectively.

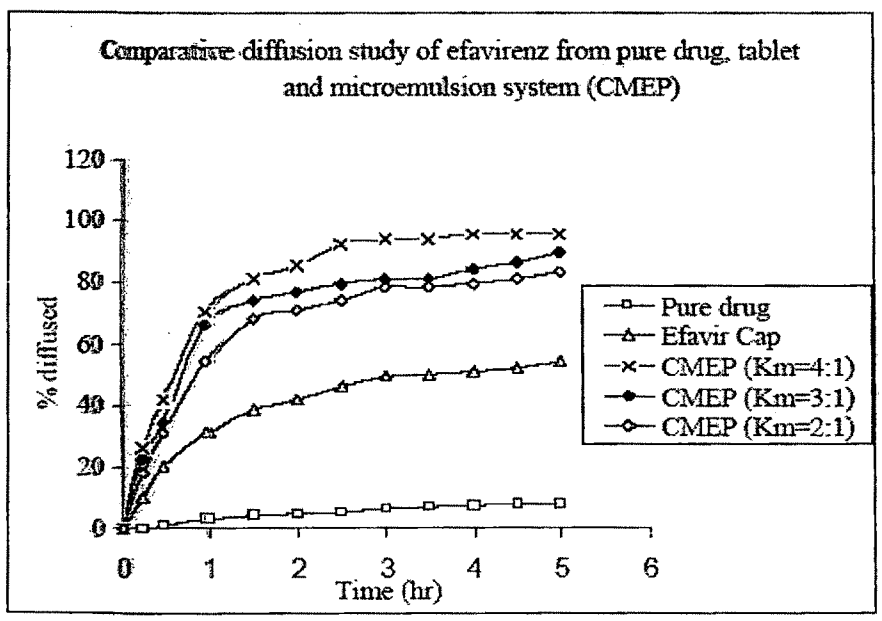

Figure 5.17: *In-vitro* diffusion profile of acyclovir through rat intestine from microemulsion (C-MEP)

It was also observed that maximum absorption was takes place when surfactant to cosurfactant ratio (Km) was 4:1 as compared to Km=3:1 or 2:1. For this reason, *in-vivo* study was carried out using Km=4:1 only.

Table 5.20 summarizes the mean flux rate (J), permeation constant (P) and diffusion coefficient (D) of pure drug, tablet and microemulsion formulation. Formulations exhibited higher flux value (J), higher permeation coefficient (P) as well as higher

diffusion coefficient (D) across the membrane compared to the other formulation, which may be due to either higher release rate from the formulation or higher retention of the drug inside the membrane resulting in lower concentration in the receptor compartment following absorption. Formulation CMEP (4:1) shows higher flux rate and higher permeation constant compare to the other formulation.

Table 5.20: Comparative diffusion parameter of acyclovir from pure drug, capsule and system C

Formulation	J(μg cm^{-2} min^{-1})	P (cm min^{-1})	D (cm^{-2} min^{-1})
Pure drug	7.43308	0.001858	1.10E-07
Efavir Cap	59.829	0.014957	4.80E-06
CMEP (4:1)	**117.8**	**0.02945**	**1.49E-05**
CMEP (3:1)	104.31	0.26078	1.32E-05
CMEP (2:1)	96.977	0.024244	1.14E-05

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

Figure 5.18 shows the in vitro diffusion study of efavirenz from system D through rat intestine. From the above *in-vitro* diffusion study it was observed all the microemulsion formulation shows greater absorption as compared to the pure drug and tablet formulation through rat intestine (Figure 5.17 and Figure 5.18). It was also observed that maximum absorption takes place when Km =2:1 as compared to when Km=3:1 or 1:1 for system D. From Figure 5.18 it was observed that, after 5hr of diffusion study only 8.2% and 54.2% of drugs get diffused from pure drug suspension and capsule suspension respectively but from Figure 5.18 it was observed that at similar time, 85.2%, 96.4% and 87.4% of efavirenz get diffused when surfactant to cosurfactant ratio (Km) was 3:1, 2:1 and 1:1 respectively.

It was also observed that similar and greater absorption was takes place when surfactant to cosurfactant ratio (Km) was 2:1 as compared to when Km = 3:1 or 1:1. For this reason, *in-vivo* study was carried out using when Km=2:1 only.

Table 5.21 summarizes the mean flux rate (J), permeation constant (P) and diffusion coefficient (D) of pure drug, tablet and microemulsion formulation. Formulations exhibited higher flux value (J), higher permeation coefficient (P) as well as higher diffusion coefficient (D) across the membrane compared to the other formulation, which may be due to either higher release rate from the formulation or higher retention of the drug inside the membrane resulting in lower concentration in the receptor compartment following absorption. Formulation DMEP (2:1) shows higher flux rate and higher permeation constant compare to the other formulation.

Table 5.21: Comparative diffusion parameter of acyclovir from pure drug, tablet and system D

Formulation	J($\mu g\ cm^{-2}\ min^{-1}$)	P ($cm\ min^{-1}$)	D ($cm^{-2}\ min^{-1}$)
Pure drug	7.43308	0.001858	1.10E-07
Efavir Cap	59.829	0.014957	4.80E-06
DMEP (3:1)	93.301	0.023325	1.19E-05
DMEP (2:1)	**108.17**	**0.027043**	**1.52E-05**
DMEP (1:1)	98.54	0.024635	1.25E-05

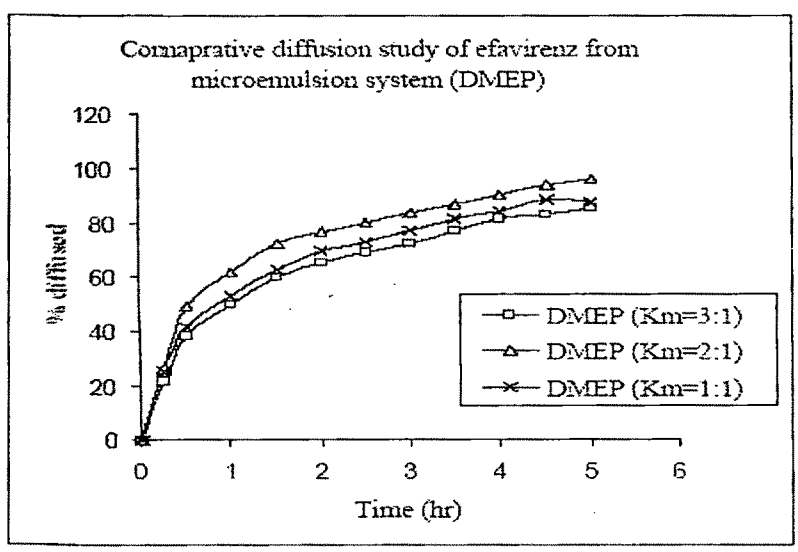

Figure 5.18: *In-vitro* diffusion profile of acyclovir through rat intestine from microemulsion (D-MEP)

5.3.9. Transmission electron microscopy (TEM):

Figure 5.19 to Figure 5.22 shows the TEM photographs of the different microemulsion sample, Form photograph it was clear that particles are of nanometer range. It was clear that particles are not completely homogenously distributed in the formulation.

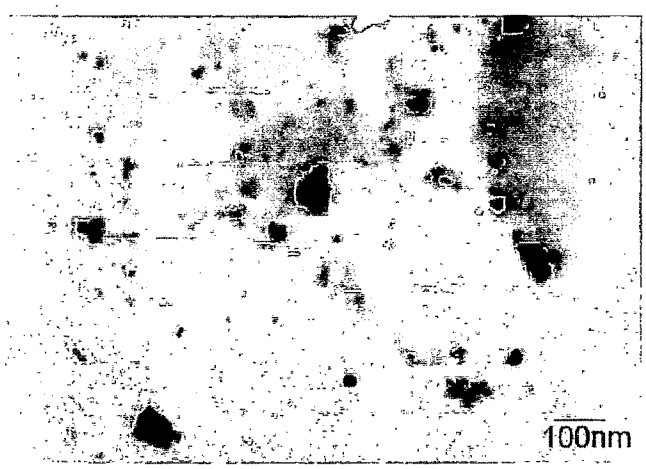

Figure 5.19: TEM photograph of system A at Km=4

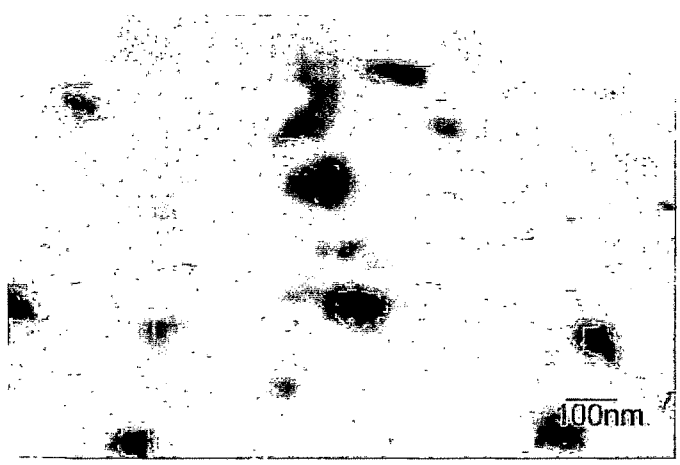

Figure 5.20: TEM photograph of system B at Km=2

Figure 5.21: TEM photograph of system C at Km=4

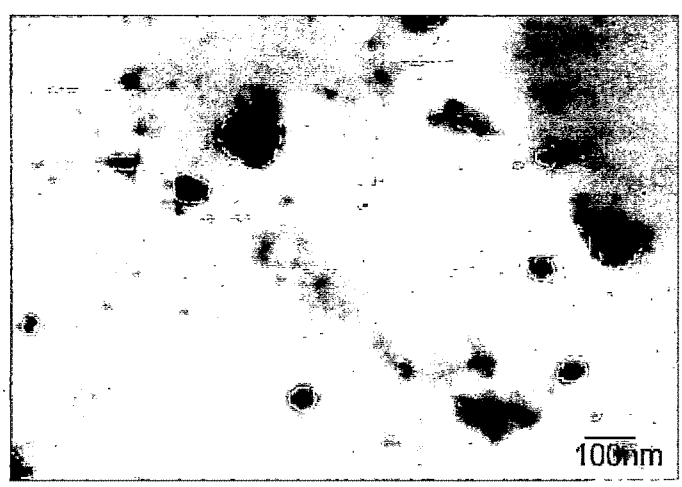

Figure 5.22 TEM photograph of system D at Km=2

5.3.10. Stability studies

Stability studies of the microemulsion samples were carried out by subjecting them to visual inspection (without stress) and centrifugation (under stress). The visual inspection test was carried out for 6 month by drawing sample at weekly interval for the first month and monthly interval for the subsequent months. The visual observations conducted show no evidence of phase separation or any flocculation or precipitation. These samples also showed no sign of phase separation under stress when subjected to centrifugation at 10000 rpm for 30 minutes or and subjected to freeze thaw cycle.

Table 5.22: Particle size (nm) (P.I*) change on stability:

Storage Temp	25 ± 2^0C			40 ± 2^0C		
Batch No	1M	3M	6M	1M	3M	6M
ME04	29.46	35.64	42.65	36.84	64.85	89.57
	(0.38)	(0.33)	(0.42)	(0.40)	(0.44)	(0.48)
ME11	18.87	24.75	36.44	32.25	48.65	68.14
	(0.24)	(0.28)	(0.32)	(0.34)	(0.32)	(0.42)
ME13	23.42	24.65	32.56	36.25	51.25	74.25
	(0.18)	(0.21)	(0.24)	(0.24)	(0.28)	(0.38)
ME23	28.25	34.56	47.34	44.65	63.52	79.65
	(0.17)	(0.19)	(0.26)	(0.24)	(0.44)	(0.38)

* Polydispersity Index (P.I.)

Table 5.22 summarizes the particle size changes during storage at different temperature. It was observed from the table that particle size was increases upon storage. The particle size of ME03 was increases up to 42.65nm and 89.57nm after six month storage at 25 ± 2^0C and 40 ± 2^0C respectively. Similarly particle size increase to 36.44, 32.56 and 47.34nm after six month storage at 25 ± 2^0C for ME11, ME13 and ME23 respectively. Particle size of the microemulsions was more increases at higher temperature (40 ±2^0C). The particle size increases to 68.14, 74.25 and 79.65nm after six month storage at 40 ± 2^0C for ME11, ME13 and ME ME23. All the microemulsions show particle size less than

100nm after six month storage. So it can be concluded that the microemulsion sample can be stored at roomtemperature for six months without drastic change in particle size.

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