Chapter 4
Formulation
&
Characterization

4.1 Introduction

Microemulsions (ME) are thermodynamically stable systems that are broadly categorized into three types. 1. oil-in-water (o/w) ME 2. water-in-oil (w/o) ME 3. Bicontinuous ME. Many researchers in various literatures have reported the formulation techniques for ME. These techniques are mainly pseudo ternary diagram construction and titration method (Lawrence and Rees 2000). Regardless of the type of ME systems, MEs can be formulated easily by mixing the oil component with surfactant and cosurfactant components. Aqueous components can be added gradually to the mixture of oil containing surfactant and co surfactant components. Since MEs are thermodynamically stable systems, they undergo spontaneous formation, facilitated by micelle formation without input of external energy into the system. Ternary phase diagram is a very important tool to study the phase behavior of the ME system. Ternary phase diagram can be represented in a triangular format, in which each coordinate represents one component of ME with 0-100% concentration in the increment of 10%. If four or more components are investigated for ME system, binary mixtures like surfactant/cosurfactant or oil/drug are taken in the ordinates and pseudo ternary phase diagram will be constructed. The advantages associated with titration techniques are rapid, reasonably accurate and precise. Economical due to limited number of trial batches. The major disadvantage is that it can provide the true picture of the phase boundary between the polyphasic and monophasic region. But within the monophasic region, the different types o/w, w/o and bicontinuous ME cannot be identified from the phase diagram which is constructed on the basis of titration method without further characterization.(Lawrence and Rees, 2000)

4.2 Materials and Instruments

Materials

- Halobetasol propionate (HP) was gifted by Lyka Ltd., Ankleshwar, Gujarat and Tacrolimus (Tac) was gifted by Cadila Pharma, Ahmedabad, Gujarat
- Capmul MCM C8, Capmul MCM L8, Capmul GMO – 50EP/NF, Lauroglycol 90, Captex 355 EP/NF, Acconan CC6, gifted by Abitec Corporation Limited, Janesville, USA.
- Labrafac PG, Labrafac Lipophile WL 1349, Capryol 90, Labrafil M1944 Cs, Transcutol, were gifted by Colorcon India, Gattefosse, France.
- Ethyl Oleate, Oleic acid, Tween 80, Tween 20, PEG 200, PEG 400, Propylene Glycol, Isopropyl Palmitate, Isopropyl Myristate were purchased from SD fine Chemicals.
- Other chemicals were of analytical grade and purchase from Sd fine chemicals, Mumbai.

Instruments:

The instruments used for the preparation, characterization and estimation of drugs in the formulation include UV-Visible Spectrophotometer, pH meter, Bath sonicator, analytical balance, magnetic stirrer, Brookfeild Viscometer, Centrifuge, Zeta sizer, Abbe Refractometer, Transmission Emission Microscope.

4.3 Methods

4.3.1 Solubility Determination

Solubility of drugs HP and Tac was determined in different oils, surfactants and cosurfactants. Drugs were added in excess to different oils, surfactants and cosurfactants and shaken by mechanical shaker for 24 hours. The samples

were allowed to stand overnight and centrifuged at 8000 rpm for 10 minutes and the drug content in the supernatant was analysed after proper dilution as described in analytical section respectively. The drug solubilities were calculated and tabulated in Table 4.1 and 4.2 for HP and Tac respectively.

4.3.2 Preparation of Microemulsion

Construction of phase diagram:

The pseudo ternary phase diagram of oil/surfactant/cosurfactant was developed by the water titration method. Aliquots of each surfactant and cosurfactant mixture (S_{mix}) were mixed with the oil at ambient temperature. For each phase diagram, the ratio of oil to the S_{mix} was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (v/v). Water was added drop wise to each oil- S_{mix} mixture under continuous stirring. After equilibrium, the samples were visually checked and determined as being clear MEs. No heating was done during the preparation. Phase diagrams were constructed using Chemix 3.5 software. The phase diagram with different ratios of surfactant and co-surfactant with different oils were constructed to explore the ME region. The area of the monophasic region was used as a tool for the selection of suitable surfactant and co surfactant mixture. Based on the solubility study, different systems were studied and listed below.

For HP

- Capmul MCM L8, Tween 80 + Transcutol P, Distilled water
- Isopropyl Myristate, Tween 80 + Transcutol P, Distilled water
- Capmul MCM L8, Tween 80 + (Transcutol P: PEG 200), Distilled water

For Tac

- Capmul MCM C8, Tween 80 + Transcutol P, Distilled water
- Capmul MCM C8, Tween 80 + Soluphor P, Distilled water
- Ethyl Oleate, Tween 80 + Transcutol P, Distilled water

Preparation of drug loaded ME:

Based on the phase diagram, the optimum S_{mix} ratio was selected and the drug loaded MEs were prepared by dissolving the drug in small increments in the oil- S_{mix} mixture then titrated with the continuous phase. The external phase was added in a drop wise manner under continous stirring. The process was optimized for the duration of stirring.

4.3.3 Optimization of ME preparation

Experimental design (3^2) was applied in the formulation of ME by varying concentrations/ levels of oil and S_{mix} and measuring globule size (GS) and zeta potential (ZP) as the responses. Nine batches of MEs of each system were prepared by titration method according to experimental design. The prepared batches were evaluated for zeta potential and particle size. The factorial design of HP ME systems 1, 2 and 3 are shown in the Tables 4.3, 4.5 & Table 4.7 respectively. Similarly the factorial design of Tac ME systems 1, 2 and 3 are shown in the Tables 4.11, 4.13 & Table 4.15 respectively.

Mathematical modeling of the preparation of ME, multiple regression analysis was carried out by using Eq. 1 to obtain a second order polynomial equation.

$$Y = b0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Where Y is the dependent variable (ZP or GS) while bi and bij represent the regression coefficients for second order polynomial and Xi represents the levels of the independent formulation variables i.e., Oil content (X_1) and Surfactant concentration(X_2). A full model was established for all the systems. Response surface plots and contour plots were plotted to study the influence of oil and S_{mix} on zeta potential and globule size. The optimized batches were

selected on the basis of higher zeta potential value, smaller particle size and safety considerations.

A check point experiment was performed to confirm the utility of polynomial equation and established contour plots in the preparation of ME. Two values of independent variables X_1 and X_2 were taken and the values of ZP and GS (dependent variable) were calculated by substituting the values in the polynomial equation. MEs were prepared experimentally by taking the amounts of the independent variables X_1 and X_2 on the same checkpoints. Each batch was prepared three times and mean ZP and GS values were determined. The check point batches of HP formulations were prepared and recorded in Table 4.6, 4.8 & 4.10. Similarly the check point batches of Tac containing formulations were prepared and recorded in Table 4.12, 4.14 & 4.16.

4.3.4 Preparation of Cetomacrogol Cream Base and incorporation of microemulsion

Cetomacrogom cream base was prepared as per British pharmaceutical codex with some modifications. The formula is as shown below:

Ingredient	**Quantity (g) /100g of cream base**
Cetostearyl alcohol	7.2
Cetomacrogol 1000	1.8
White petroleum Jelly	13.5
Isopropyl myristate	3.75
Isopropyl palmitate	3.75
Chlorocresol	0.1
Propylene glycol	5
Purified water	Q.S. to make 100g

Incorporation of drug loaded ME into cetomacrogol cream base

The drug loaded ME was incorporated into cream base by replacing an equivalent volume of water from cream base so as to give a final concentration of 0.035% and 0.05% of HP and 0.1% Tac in cream. ME was added into cream base when the temperature is not more than 30°C, mixed gently and allowed to stand overnight.

4.3.5 Characterization

Dilution test: Dilution tests are based on the fact that the emulsion is only miscible with the liquid that forms its continuous phase. The system is diluted with either the oil or the aqueous phase, which ever is used in the ME preparation. Hence, in case of o/w system the ME can be diluted with the aqueous phase while with w/o ME the system is diluted with the oil used.

Globule size determination:

The globule size determination (Kaler and Prager 1982; Roland et al 2003) of MEs were determined using photon correlation spectroscopy (PCS) with in-built Zetasizer (model: Nano ZS, Malvern isnstruments, UK) at 633nm. The globule size was measured with Malvern zetasizer. The instrument is based on the principle of dynamic light scattering (DLS), also sometimes referred to as photon correlation spectroscopy or quasi elastic light scattering. DLS is a technique of measuring the size of particles typically in the sub-micron region and is usually applied to the measurement of particle suspended within a liquid. The technique measures particle diffusion due to Brownian motion and relates this to the size of the particles. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them. The parameter calculated is defined as the translational diffusion coefficient. The particle size is then calculated from the translational diffusion coefficient using the Strokes-Einstein equation and recorded.

Measurement conditions for zeta potential and globule size were optimized by measuring zeta potential and globule size for the dispersions of different dilutions. The dilution of the ME was made in such a way that the integrity of the globules were maintained with sufficient inter particle space and minimal multiple light scattering during measurement.

Zeta potential determination:

Malvern Zetasizer Nano ZS was used to measure the zeta potential of the globules based on the electrophoresis and electrical conductivity of the formed ME. The electrophoretic mobility (μm/s) of the particles was converted to the zeta potential by in-built software based on Helmholtz-Smoluchowski equation. Measurements were performed using small volume disposable zeta cell. Average of twenty measurements of each sample was used to derive the average zeta potential.

Transmittance: The % transmittance of ME was checked against distilled water with UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan) at 630nm.

pH: pH of the formulations were measured using pH meter (Labindia).

Assay: Assay of the MEs and MEC was determined as per the methods described in the analytical section and the results were recorded in Tables 4.9, 4.10 & 4.17, 4.18.

Viscosity: Viscosity of the formulations were determined using Brookfield cone and plate Rheometer (Model LVDV III) using CPE spindle at the rotational speed of 5rpm, shear rate of 10 at 25±1°C and the results were recorded in Tables 4.9, 4.10 & 4.17, 4.18.

Refractive Index: Refractive index of the placebo ME and drug loaded ME were determined using an Abbe type thermostated refractometer.

Transmission Electron Microscopy (TEM)

TEM is used as a tool to study the morphology and structure of the delivery systems. The TEM images of MEs were taken to get idea about the size of MEs (Sheikh Shafiq et al 2007). The images were taken Tecnai20 with CCD camera operating at 200kV (Philips Instruments, Holland) and capable of point to point resolution. To perform TEM observations, a drop of diluted (1 in 10 dilution) ME was directly deposited on the copper grid and observed after drying and the positive images are shown in Fig 4.23 & Fig 4.24. The cream base was imaged after dilution and negative staining with 2% phosphotungstic acid. The cream base was also imaged after incorporation of drug loaded ME and suitable dilution. The images are shown in fig 4.25, 4.26 and 4.27.

4.3.6 Stability Studies

The stability of the micro emulsion was assessed by conducting long term stability study and accelerated stability studies.

4.3.6.1 Long term stability study

In long term stability study, the MEs were packed in the borosil screw capped vials and were kept at room temperature (25-35°C) and refrigeration temperature (2-8°C). Over the time period micro emulsion systems were assessed for their zeta potential, globule size, physical stability, assay and pH.

Zeta potential measurement: Zeta potential of the MEs were determined at predetermined time interval and the results recorded in Tables 4.20 & 4.21

Globule size determination: Particle size of the MEs were determined at predetermined time interval and recorded in Tables 4.20 & 4.21

Physical stability: During the storage period, the MEs were visualized for any precipitation of drug, creaming, phase separation or flocculation. Stability on dilution and %transmittance of the samples were measured as an indicator of the physical stability of the ME system and recorded in Tables 4.20 & 4.21

% Assay: The drug content of the formulations were determined as per the method described in analytical section and recorded in Tables 4.20 & 4.21

pH: pH of ME formulations were monitored during the storage period and recorded in Tables 4.20 & 4.21

4.3.6.2 Accelerated stability study

Accelerated stability studies are the essential tools to study the thermodynamic stability of micro emulsions (Sheikh Shafiq et al 2007; Nornoo et al 2008).

- The formulations were centrifuged for 30 minute at 10,000 rpm and observed for phase separation.
- The systems were kept for freeze/ thaw cycles between - 21ºC and 25ºC for not less than 48 hours at each stage for three cycles.
- The systems were subjected to 6 cycles of heating / cooling cycle by keeping them at 4ºC and 45ºC for not less than 48 hours at each stage.
- The formulations were observed for zeta potential, globule size and %transmittance before and after the centrifugation, freeze thaw cycle and heating cooling cycle.

4.4 Results

4.4.1 Solubility Studies

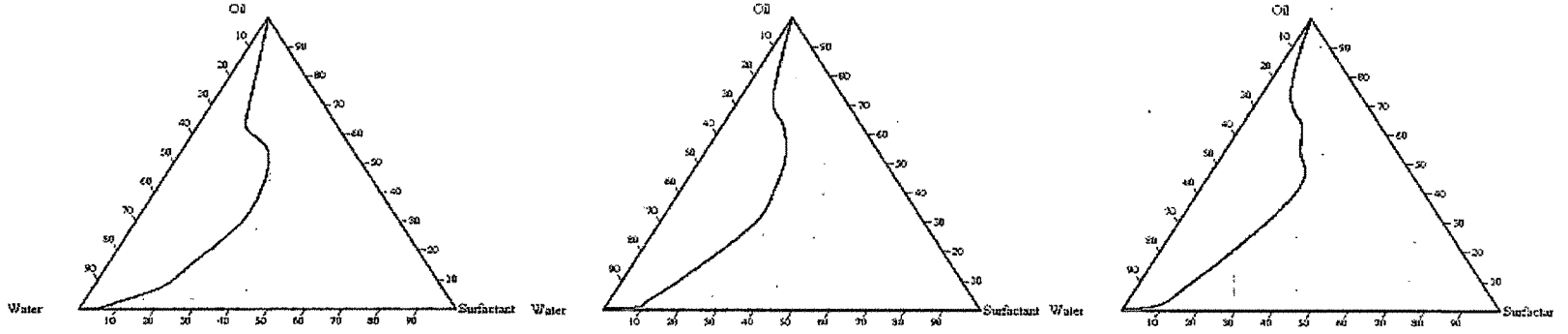
Table 4.1: Solubility of HP in excipients

Excipients	~ Drug dissolved (mg/ml)
Oleic acid	2.5
Isopropyl myristate	6.5
Ethyl Oleate	2.2
Miglyol 812	5
Labrafac lipophile WL 1349	3
Capmul MCM C8	8
Capmul MCM L8	10
Tween 80	35
Cremophore EL	15
Cremophore RH 40	17
Transcutol P	>90
Soluphor P	>60
Decanol	1
Butanol	30
Propylene glycol	6
PEG 200	>80
Imwitor 380	0.6
Labrasol	20

Table 4.2: Solubility of Tac in excipients

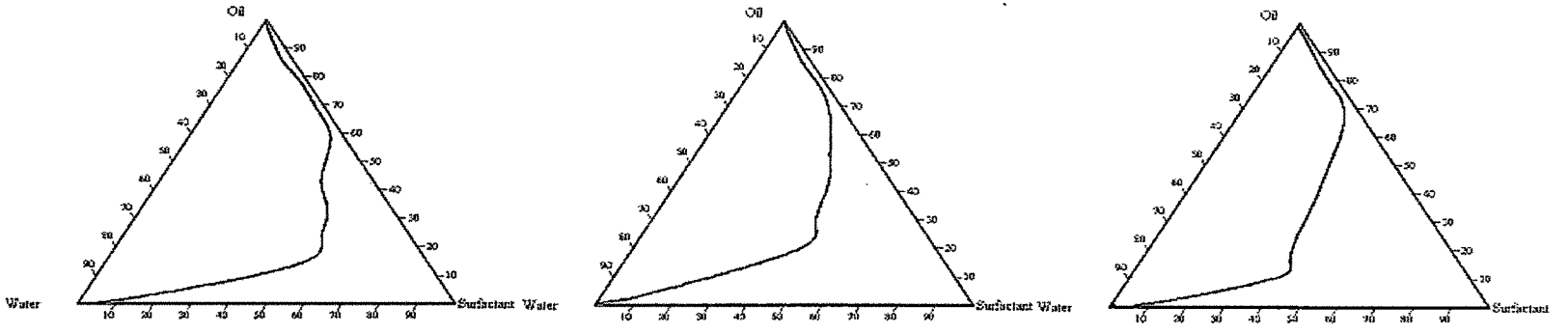
Excipients	**~ Drug dissolved (mg/ml)**
Oleic acid	4
Isopropyl myristate	0.8
Ethyl Oleate	10
Miglyol 812	0.7
Labrafac lipophile WL 1349	0.8
Capmul MCM C8	30
Capmul MCM L8	24
Lauroglycol 90	10
Tween 80	20
Cremophore EL	18
Transcutol P	>150
Soluphor P	>120
Decanol	2
Butanol	3
Propylene glycol	35
PEG 200	35
Imwitor 380	8

4.4.2 Preparation Optimization and Characterization of Halobetasol Propionate Microemulsion



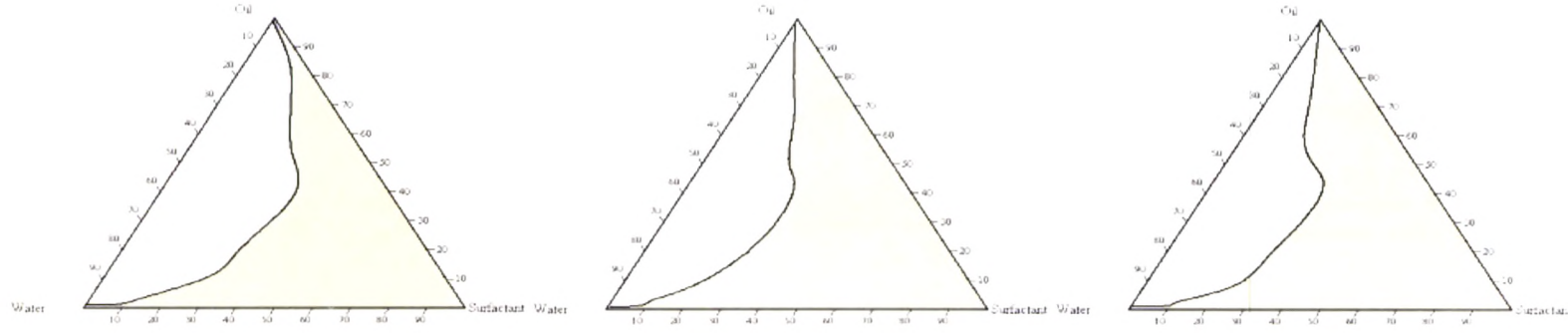
(Tween 80 : Transcutol p :: 1 : 1) (Tween 80 : Transcutol p :: 2 : 1) (Tween 80 : Transcutol p :: 3 : 1)

Fig. 4.1: Phase diagram of HP system 1 (Capmul MCM L8, Tween 80 + Transcutol P, Distilled water)



(Tween 80 : Transcutol p :: 1 : 1) (Tween 80 : Transcutol p :: 2 : 1) (Tween 80 : Transcutol p :: 3 : 1)

Fig. 4.2: Phase diagram of HP system 2 (Isopropyl Myristate, Tween 80 + Transcutol P, Distilled water)



(Tween 80: (Transcutol p +PEG 200) :: 1:1) (Tween 80 : (Transcutol p +PEG 200) :: 2:1) (Tween 80 : (Transcutol p +PEG 200) :: 3:1)

Fig. 4.3: Phase diagram of HP system 3 (Capmul MCM L8, Tween 80 + (Transcutol P: PEG 200), Distilled water)

Table 4.3: 3^2 Factorial design for HP System1

Capmul MCM L8, Tween 80 + Transcutol P, Distilled water (1:1)

Formulation	% v/v Oil	% v/v S_{mix}	Zeta potential * (mV)	Globule size* (nm)	%Transmittance (630nm)	
					Before Dilution	Dilution (1 in 10)
A1	2.5	25	-12.5 ± 1.2	12.3 ± 2.3	>99%	>99%
A2	2.5	32.5	-12.6 ± 0.9	13.4 ± 1.9	>99%	>99%
A3	2.5	40	-11.2 ± 0.7	10.7 ± 2.0	>99%	>99%
A4	5	25	-7.7 ± 0.8	18.5 ± 2.7	>99%	>99%
A5	5	32.5	-6.47 ± 1.4	15.4 ± 1.6	>99%	>99%
A6	5	40	-7.38 ± 0.5	14.7 ± 1.4	>99%	>99%
A7	7.5	25	4.92 ± 0.4	166.0 ± 9.6	82.3%	TOD
A8	7.5	32.5	-4.38 ± 0.3	116.3 ± 6.5	88.5%	TOD
A9	7.5	40	-5.52 ± 0.7	23.6 ± 3.1	>99%	>99%
Optimization batches						
A10	3.4	25	-9.0 ± 1.5	16.2 ± 2.1	>99%	>99%
A11	3.4	32.5	-11.1 ± 1.8	13.6 ± 2.6	>99%	>99%
A12	3.75	25	-9.19 ± 0.9	15.2 ± 1.6	>99%	>99%
A13	3.75	32.5	-8.46 ± 0.7	14.5 ± 1.3	>99%	>99%
A14	**3.75**	**30**	**-10.8 ± 1.9**	**14.8 ± 1.1**	**>99%**	**>99%**
A15	2	25	-12.6 ± 0.8	14.0 ± 1.8	>99%	>99%
A16	4.5	25	-6.78 ± 1.1	18.1 ± 2.6	>99%	>99%
A17	7	25	-5.7 ± 0.8	147.1 ± 4.6	83.5%	TOD
A18	2	40	-10.1 ± 0.6	12.0 ± 1.2	>99%	>99%
A19	4.5	40	-6.48 ± 0.4	17.0 ± 1.3	>99%	>99%
A20	7	40	-4.5 ± 0.5	17.8 ± 2.3	>99%	>99%

* measured for the dispersion of 1 in 10 dilution. TOD - Turbid on dilution

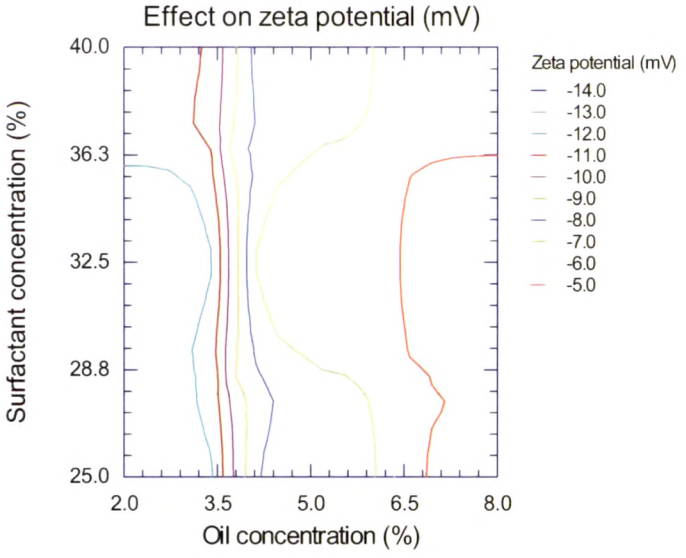


Fig 4.4: Contour plot for zeta potential of HP system1

Y1 = -6.9256 + 3.58 **X1** + 0.17 **X2** - 1.337 **X11** - 0.387 **X22** - 0.475 **X12**

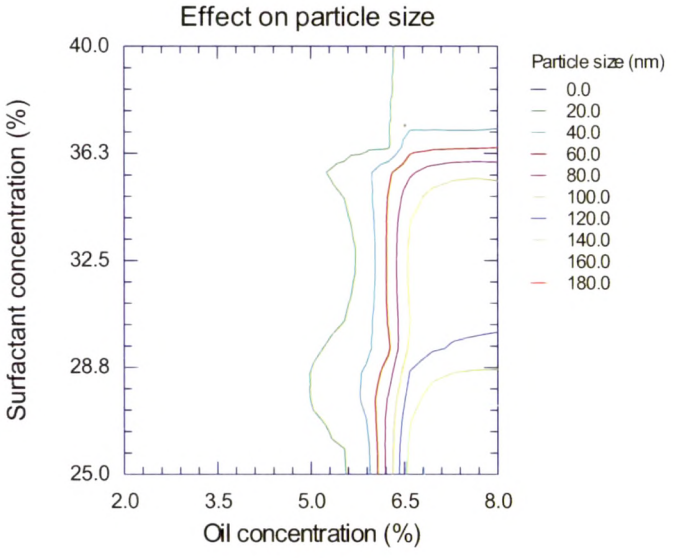


Fig 4.5: Contour plots for globule size of HP system1

Y2 = 21.211 + 45.033 **X1** – 24.75 **X2** + 40.733 **X11** – 7.517 **X22** – 35.025 **X12**

Where

Y2 - globule size

Y1 - Zeta potential,

X1 - Oil concentration

X2 - S_{mix} concentration,

X11 - Main effect of oil

X22 - Main effect of S_{mix},

X12 - interaction effect of oil and S_{mix}

Table 4.4: Checkpoint batches for HP system1

% v/v Oil	% v/v S_{mix}	Predicted Zeta Potential (mV)	Experimental Zeta potential (mV)	Predicted Size (nm)	Experimental size (nm)
2	25	-12.5	-12.6* ± 0.8	12.3	14.0* ± 1.8
4.5	25	-6.5	-6.78* ± 1.1	15.4	18.1* ± 2.6
7	25	-4.9	-5.7* ± 0.8	164.9	147.1* ± 4.6
2	40	-11.2	-10.1* ± 0.6	10.0	12.0* ± 1.2
4.5	40	-7.4	-6.48* ± 0.4	14.7	17.0* ± 1.3
7	40	-5.5	-4.5* ± 0.5	23.6	17.8* ± 2.3

*Difference between predicted and experimental values were found to be insignificant (P>0.05)

** n = 3

Table 4.5: 3^2 Factorial design for HP System 2

Isopropyl Myristate, Tween 80 + Transcutol P, Distilled water (2:1)

Formulation	**% v/v Oil**	**% v/v S_{mix}**	**Zeta potential * (mV)**	**Globule size* (nm)**	**%Transmittance (630nm)**	
					Before Dilution	**Dilution (1 in 10)**
B1	2.5	25	-10.8 ± 1.9	19.3 ± 1.9	>99%	>99%
B2	2.5	32.5	-9.49 ± 1.3	14.2 ± 1.4	>99%	>99%
B3	2.5	40	-10.3 ± 1.7	12.4 ± 2.2	>99%	>99%
B4	5	25	-5.79± 1.4	138.6 ± 8.4	79.6%	TOD
B5	5	32.5	-7.08 ± 1.3	109.2 ± 10.2	84.9%	TOD
B6	5	40	-7.8 ± 1.5	19.56 ± 1.9	>99%	>99%
B7	7.5	25	-6.29 ± 0.9	145.6± 9.9	81.3%	TOD
B8	7.5	32.5	-8.8 ± 1.2	138.3 ± 13.5	86.5%	TOD
B9	7.5	40	-4.35 ± 0.6	238.1 ± 20.1	39%	TOD
Optimization batches						
B10	3.0	27	-10.2 ± 2.1	15.2 ± 1.1	>99%	>99%
B11	3.0	33	-8.7 ± 1.5	12.3 ± 1.6	>99%	>99%
B12	3.4	32	-8.6 ± 1.9	12.5 ± 2.6	>99%	>99%
B13	**3.4**	**37**	**-10.1 ± 2.5**	**11.4 ± 1.3**	**>99%**	**>99%**
B14	3.75	37	- 9.8 ± 1.9	21.3 ± 3.6	>99%	>99%
B15	2	25	-12.1 ± 2.5	11.8 ± 2.8	>99%	>99%
B16	2	32	-11.5 ± 1.8	15.8 ± 3.6	>99%	>99%
B17	4.5	32	-6.3 ± 0.9	74.3 ± 8.6	93%	TOD
B18	4.5	40	-9.4 ± 0.8	16.1 ± 1.2	>99%	>99%
B19	7	25	-5.1 ± 0.6	170.1 ± 11.3	44%	TOD
B20	7	40	-6.2 ± 1.1	158.0 ± 12.2	64%	TOD

* measured for the dispersion of 1 in 10 dilution. TOD - Turbid on dilution

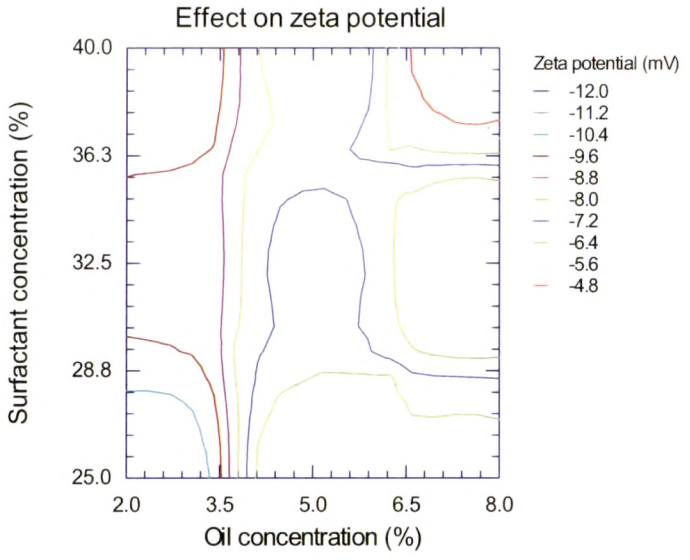


Fig 4.6: Contour plot for zeta potential of HP system2

Y1 = -7.491 + 1.858 **X1** + 0.072 **X2** – 1.448 **X11** + 0.902 **X22** +0.36 **X12**

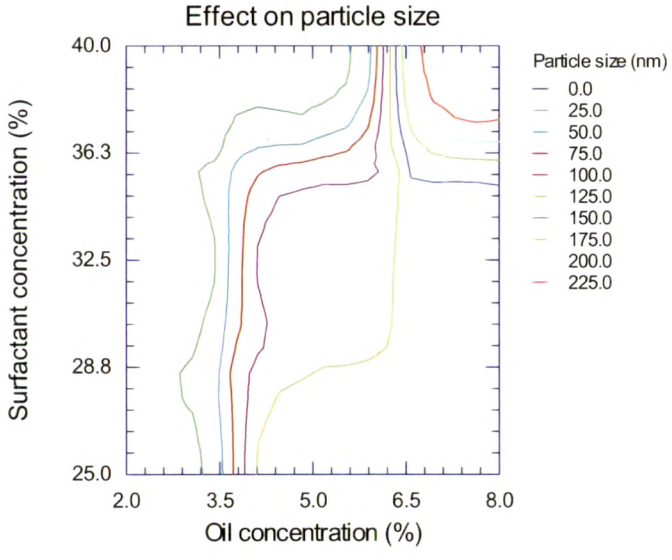


Fig 4.7: Contour plot for globule size of HP system2

Y2 = 83.436 + 79.342 **X1** – 5.59 **X2** + 5.57 **X11** + 8.427 **X22** + 24.825 **X12**

Table 4.6: Checkpoint batches for HP system 2

% v/v Oil	% v/v S_{mix}	Predicted Zeta Potential (mV)	Experimental Zeta potential (mV)	Predicted Size (nm)	Experimental size (nm)
2	25	-10.8	-12.1* ± 2.5	19.4	11.8* ± 2.8
2	32	-9.5	-11.5* ± 1.8	14.2	15.8* ± 3.6
4.5	32	-7.1	-6.3* ± 0.9	108.4	74.3* ± 8.6
4.5	40	-7.8	-9.4* ± 0.8	19.7	16.1* ± 1.2
7	25	-6.3	-5.1* ± 0.6	145.5	170.1* ± 11.3
7	40	-8.7	-6.2* ± 1.1	138.2	158.0* ± 12.2

*Difference between predicted and experimental values were found to be insignificant ($P>0.05$) ** n = 3

Table 4.7: 3^2 Factorial design for HP System 3 (Capmul MCM L8, Tween 80 + (Transcutol P: PEG 200(1:1)), Distilled water) (2:1)

Formulation	% v/v Oil	% v/v S_{mix}	Zeta potential * (mV)	Globule size* (nm)	%Transmittance (630nm)	
					Before Dilution	Dilution (1 in 10)
C1	2.5	25	-5.6 ± 1.6	17.3 ± 2.3	>99%	>99%
C2	2.5	32.5	-6.4 ± 1.8	13.1 ± 1.5	>99%	>99%
C3	2.5	40	-7.8 ± 2.3	14.7 ± 1.2	>99%	>99%
C4	5	25	-6.1± 1.4	23.9 ± 4.2	>99%	>99%
C5	5	32.5	-7.5 ± 1.9	20.3 ± 1.2	>99%	>99%
C6	5	40	-10.5 ± 2.5	20.1 ± 2.9	>99%	>99%
C7	7.5	25	-2.2 ± 0.5	243.5± 13.9	23%	TOD
C8	7.5	32.5	-3.4 ± 1.2	166 ± 11.5	76.5%	TOD
C9	7.5	40	-3.3 ± 0.4	126.3 ± 18.1	79%	TOD
Optimization batches						
C10	3	28	-8.9 ± 2.4	16.2 ± 1.7	>99%	>99%
C11	3.5	28	-9.4 ± 1.5	16.8 ± 1.9	>99%	>99%
C12	**4**	**28**	**-9.6 ± 1.8**	**18.6 ± 2.7**	**>99%**	**>99%**
C13	3	33	-7.1 ± 1.5	14.5 ± 1.5	>99%	>99%
C14	4	33	-5.3 ± 1.2	14.9 ± 3.7	>99%	>99%
C15	2	25	-6.3 ± 1.5	18.6 ± 1.8	>99%	>99%
C16	2	40	-8.2 ± 2.8	13.9 ± 2.6	>99%	>99%
C17	4.5	32	-6.3 ± 0.5	16.4 ± 2.6	>99%	>99%
C18	4.5	40	-7.6 ± 0.9	18.3 ± 2.2	>99%	>99%
C19	7	25	-3.9 ± 0.3	228.1 ± 22.3	24%	TOD
C20	7	40	-4.5 ± 1.2	19.2 ± 2.2	>99%	>99%

* measured for the dispersion of 1 in 10 dilution. TOD - Turbid on dilution

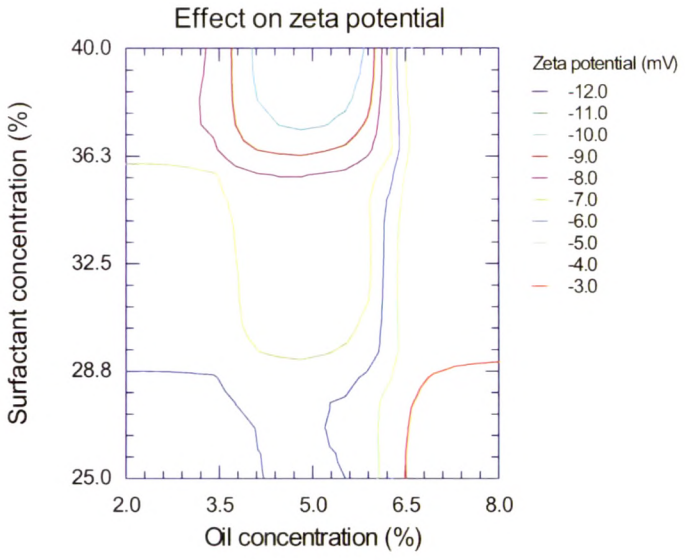


Fig 4.8: Contour plot for zeta potential of HP system 3

Y1 = -7.916 + 1.835**X1** -1.317 **X2** + 3.238**X11** - 0.147 **X22** + 0.263 **X12**

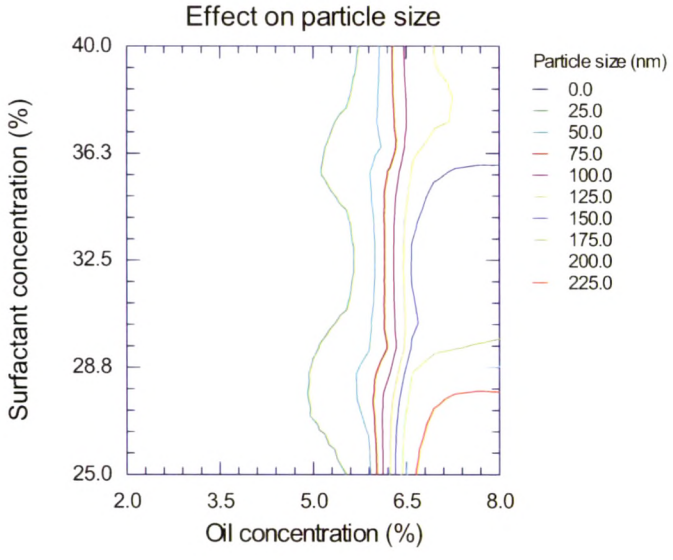


Fig 4.9: Contour plot for globule size of HP system 3

Y2 = 16.293 + 81.635 **X1** – 20.573 **X2** + 75.265 **X11** + 7.71 **X22** – 28.59 **X12**

Table 4.8: Checkpoint batches for HP system 3

% v/v Oil	% v/v S_{mix}	Predicted Zeta Potential (mV)	Experimental Zeta potential (mV)	Predicted Size (nm)	Experimental size (nm)
2	25	-5.6	-6.3 ± 1.5*	17.4	18.6 ± 1.8*
2	40	-7.8	-8.2 ± 2.8*	14.7	13.9 ± 2.6*
4.5	32	-7.5	-6.3 ± 0.5*	20.3	16.4 ± 2.6*
4.5	40	-10.5	-7.6 ± 0.9*	20.2	18.3 ± 2.2*
7	25	-2.2	-3.9 ± 0.3*	241.3	228.1 ± 22.3*
7	40	-3.4	-4.5 ± 1.2*	25.3	19.2 ± 2.2*

*Difference between predicted and experimental values were found to be insignificant (P>0.05) ** n = 3

Table 4.9: Optimized HP formulations

Test	A14	B13	C12
Zeta potential(mV)	**-10.8 ± 1.9**	-10.1 ± 2.5	-9.6 ± 1.8
Globule size(nm)	**14.8 ± 1.1**	11.4 ± 1.3	18.6 ± 2.7
%Transmittance	**>99%**	>99%	>99%
pH at 25°C	**5.4**	5.7	5.6
Drug Loading (mg/10 ml)	**25**	17.5	20
Assay (%)	**99.6 ± 1.3 %**	98.9 ± 2.4 %	99.1 ± 1.9 %
Viscosity at 25°C (cP)	**50.083 ± 0.116**	54.23 ± 0.985	51.23 ± 0.561

Table 4.10: Characterization of Halobetasol Propionate Microemulsion Based Cream (HPMEC) 0.035% and HPMEC 0.05%

Test	HPMEC 0.035%	HPMEC 0.05%
Appearance	White smooth textured	White smooth textured
pH at 25°C	5.4	5.3
Assay (%)	96.75 ± 1.62	97.23 ± 2.31
Viscosity at 25°C (KcP)	38.97 ± 2.36	42.15 ± 3.14

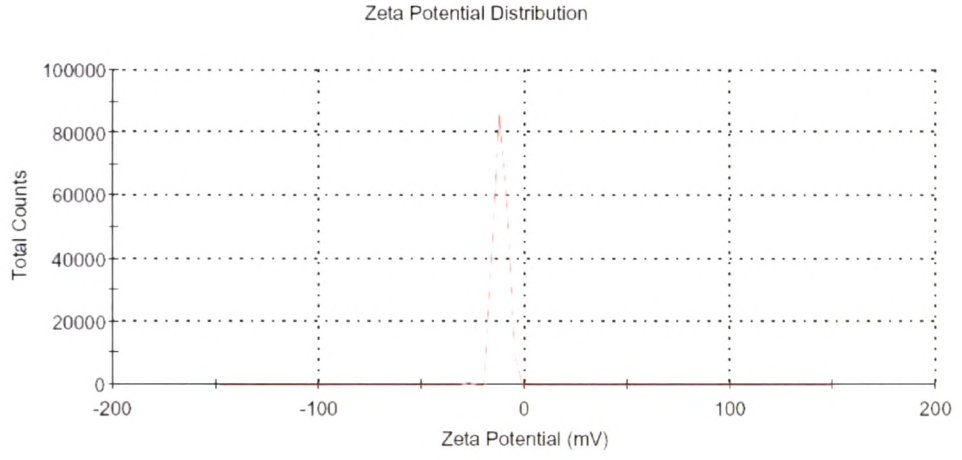


Fig 4.10: Zeta potential distribution of the optimized batch A14

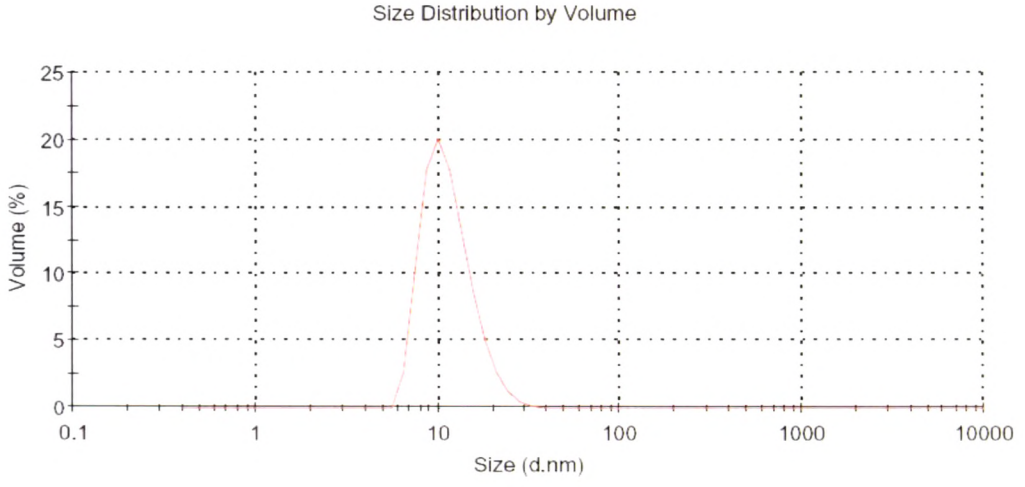
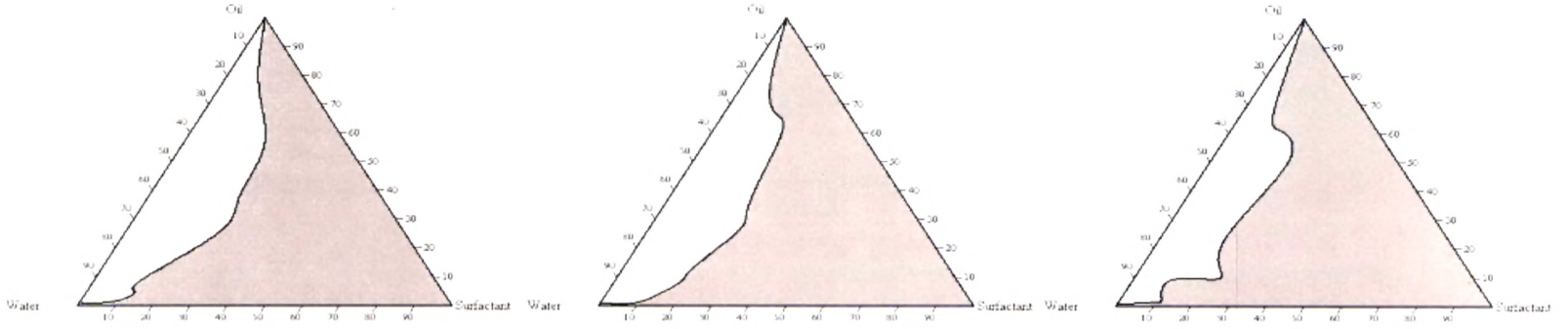


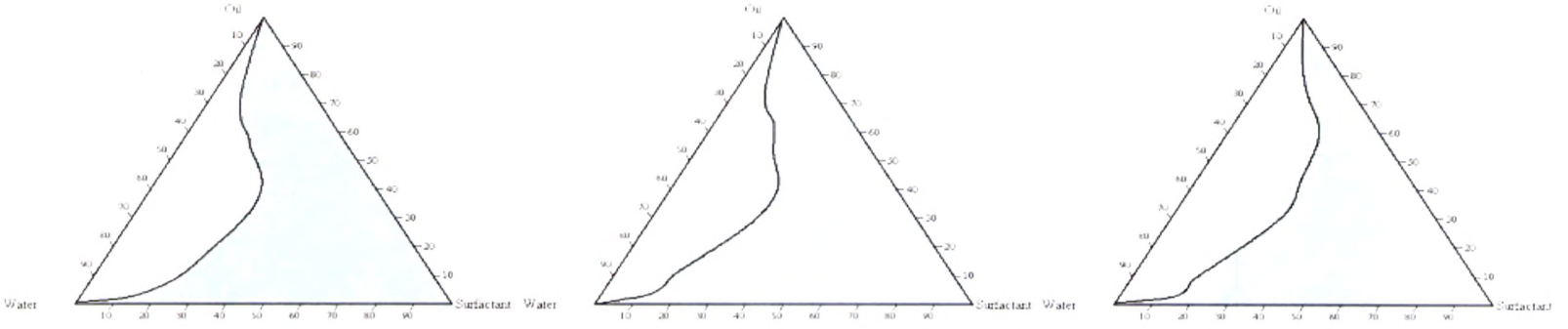
Fig 4.11: Size distribution of the optimized batch A14

4.4.3 Preparation Optimization and Characterization of Tacrolimus Microemulsion



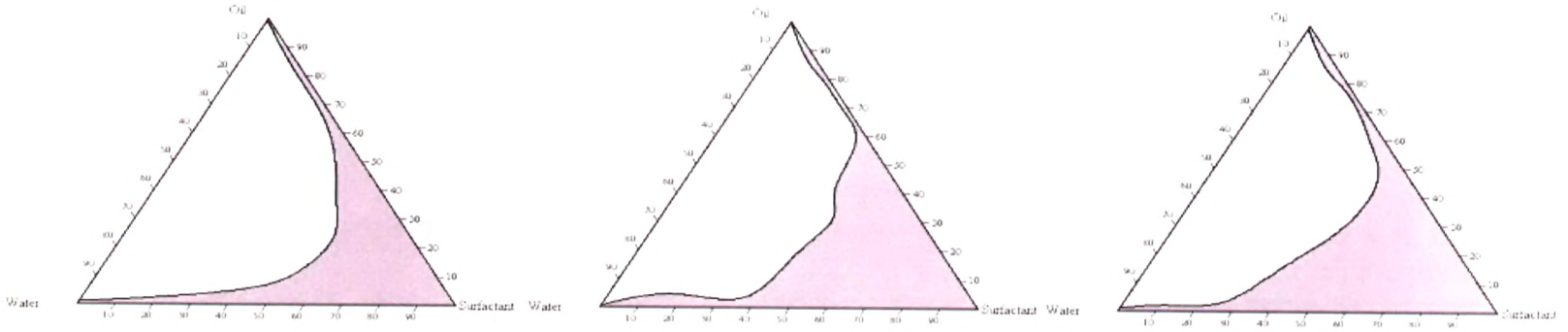
(Tween 80 : Transcutol p :: 1 : 1) (Tween 80 : Transcutol p :: 2 : 1) (Tween 80 : Transcutol p :: 3 : 1)

Fig 4.12: Phase diagram of Tac system 1 (Capmul MCM C8, Tween 80 + Transcutol P, Distilled water**)**



(Tween 80 : Soluphor p :: 0.5 : 1) (Tween 80 : Soluphor p :: 1 : 1) (Tween 80 : Soluphor p :: 2 : 1)

Fig 4.13: Phase diagram of Tac system 2 (Capmul MCM C8, Tween 80 + Soluphor P, Distilled water**)**



(Tween 80 : Transcutol p :: 1 : 1) (Tween 80 : Transcutol p :: 2 : 1) (Tween 80 : Transcutol p :: 3 : 1)

Fig 4.14: Phase diagram of Tac system 3 (Ethyl oleate, Tween 80 + Transcutol P, Distilled water**)**

Table 4.11: 3^2 Factorial design for Tac System 1

Capmul MCM C8, Tween 80 + Transcutol P, Distilled water (1:1)

Formulation	% v/v Oil	% v/v S_{mix}	Zeta potential* (mV)	Globule size* (nm)	%Transmittance (630nm)	
					Before Dilution	Dilution (1 in 10)
D1	2.5	25	-12.6 ± 1.3	12.4 ± 2.5	>99%	>99%
D2	2.5	32.5	-10.9 ± 1.4	13.3 ± 1.9	>99%	>99%
D3	2.5	40	-13.5 ± 2.4	11.4 ± 1.2	>99%	>99%
D4	5	25	- 11.2 ± 1.9	23.5 ± 3.7	>99%	>99%
D5	5	32.5	-9.8 ± 1.2	15.6 ± 1.7	>99%	>99%
D6	5	40	-10.7 ± 2.4	13.5 ± 2.2	>99%	>99%
D7	7.5	25	-7.8 ± 0.9	33.5 ± 3.9	>99%	>99%
D8	7.5	32.5	-6.9 ± 1.0	20.6 ± 1.5	>99%	>99%
D9	7.5	40	-8.2 ± 1.3	16.5 ± 1.8	>99%	>99%
Optimization batches						
D10	3	28	-11.9 ± 2.4	14.2 ± 1.7	>99%	>99%
D11	3.5	28	-10.4 ± 1.5	15.8 ± 1.4	>99%	>99%
D12	**3.75**	**30**	**-10.8 ± 1.7**	**15.9 ± 1.8**	**>99%**	**>99%**
D13	3	33	-10.1 ± 1.5	14.9 ± 1.5	>99%	>99%
D14	4	33	-8.3 ± 1.2	14.5 ± 3.7	>99%	>99%
D15	2	25	-12.1 ± 1.5	12.6 ± 1.8	>99%	>99%
D16	2	40	-12.3 ± 1.5	13.6 ± 1.8	>99%	>99%
D17	4.5	32	-9.4 ± 0.5	16.9 ± 2.2	>99%	>99%
D18	4.5	40	-9.3 ± 0.5	16.1 ± 2.6	>99%	>99%
D19	7	25	-6.9 ± 0.3	28.1 ± 3.5	>99%	>99%
D20	7	40	-8.5 ± 1.2	17.2 ± 2.2	>99%	>99%

* measured for the dispersion of 1 in 10 dilution. TOD - Turbid on dilution

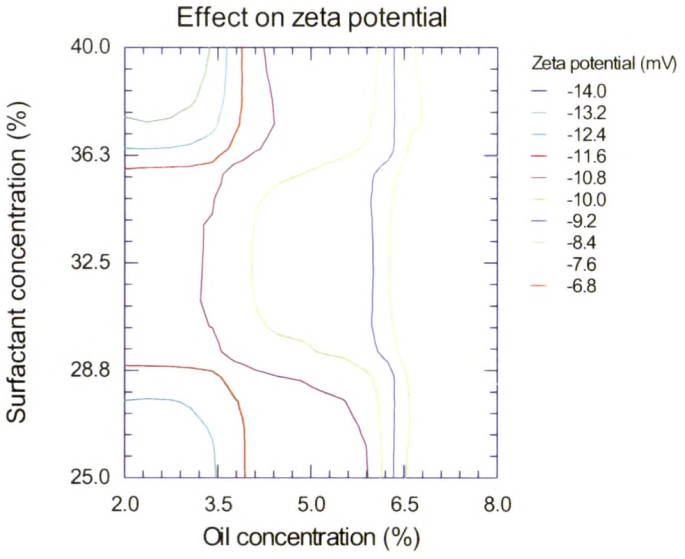


Fig 4.15: Contour plot for zeta potential of Tac system1

Y1 = -14.2502 + 4.6932 **X1** + 0.0128 **X2** - 0.0791 **X11** + 0.0018 **X22** + 0.0661 **X12**

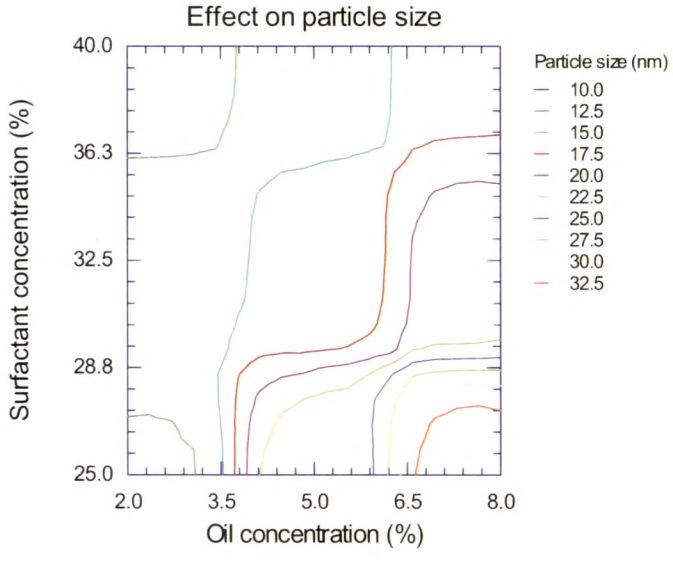


Fig 4.16: Contour plot for globule size of Tac system1

Y2 = 28.9144 + 4.408 **X1** - 0.6537 **X2** + 0.2061 **X11** + 0.0069 **X22** – 0.1014 **X12**

Table 4.12: Checkpoint batches for Tac system 1

% v/v Oil	% v/v S_{mix}	Predicted Zeta Potential (mV)	Experimental Zeta potential (mV)	Predicted Size (nm)	Experimental size (nm)
2	25	-12.63	-12.1 ± 1.5*	12.36	12.6 ± 1.8*
2	40	-13.4	-12.3 ± 1.5*	11.42	13.6 ± 1.8*
4.5	32	-9.8	-9.4 ± 0.5*	15.61	16.9 ± 2.2*
4.5	40	-10.67	-9.3 ± 0.5*	13.42	16.1 ± 2.6*
7	25	-7.82	-6.9 ± 0.3*	33.36	28.1 ± 3.5*
7	40	-8.21	-8.5 ± 1.2*	16.51	17.2 ± 2.2*

*Difference between predicted and experimental values were found tobe insignificant (P>0.05). ** n = 3

Table 4.13: 3^2 Factorial design for Tac system 2

Capmul MCM C8, Tween 80 + Soluphor P, Distilled water (1:1)

Formulation	% v/v Oil	% v/v S_{mix}	Zeta potential * (mV)	Globule size* (nm)	%Transmittance (630nm)	
					Before Dilution	Dilution (1 in 10)
E1	2.5	25	-15.6 ± 1.1	15.1 ± 2.7	>99%	>99%
E2	2.5	32.5	-15.7 ± 1.6	13.4 ± 1.7	>99%	>99%
E3	2.5	40	-17.3 ± 2.4	14.2 ± 1.6	>99%	>99%
E4	5	25	- 8.1 ± 1.8	92.3 ± 7.3	86 %	TOD
E5	5	32.5	-7.13 ± 1.5	17.0 ± 1.5	>99%	>99%
E6	5	40	-13.2 ± 2.9	14.6 ± 2.2	>99%	>99%
E7	7.5	25	-9.14 ± 0.9	104.1 ± 13.9	84 %	TOD
E8	7.5	32.5	-6.74 ± 1.2	98.2 ± 11.5	89 %	TOD
E9	7.5	40	-7.53 ± 1.5	18.5 ± 1.8	>99%	>99%

Optimization batches

E10	3.5	30	-8.7 ± 1.4	16.3 ± 1.9	>99%	>99%
E11	**4**	**30**	**-13.9 ± 2.5**	**20.4 ± 2.3**	**>99%**	**>99%**
E12	3.5	35	-11.1 ± 1.7	15.7 ± 2.5	>99%	>99%
E13	4	35	-14.8 ± 1.8	16.4 ± 2.1	>99%	>99%
E14	3.75	32	-12.3 ± 1.6	15.9 ± 2.3	>99%	>99%
E15	2	25	-14.7 ± 1.7	18.7 ± 1.9	>99%	>99%
E16	2	40	-12.9 ± 2.4	11.8 ± 3.1	>99%	>99%
E17	4.5	32	-5.9 ± 0.7	23.8 ± 2.9	>99%	>99%
E18	4.5	40	-9.9 ± 1.0	15.8 ± 2.7	>99%	>99%
E19	7	25	-6.7 ± 1.3	69.2 ± 12.3	94%	72%
E20	7	40	-4.8 ± 1.4	26.3 ± 4.2	>99%	>99%

* measured for the dispersion of 1 in 10 dilution. TOD - Turbid on dilution

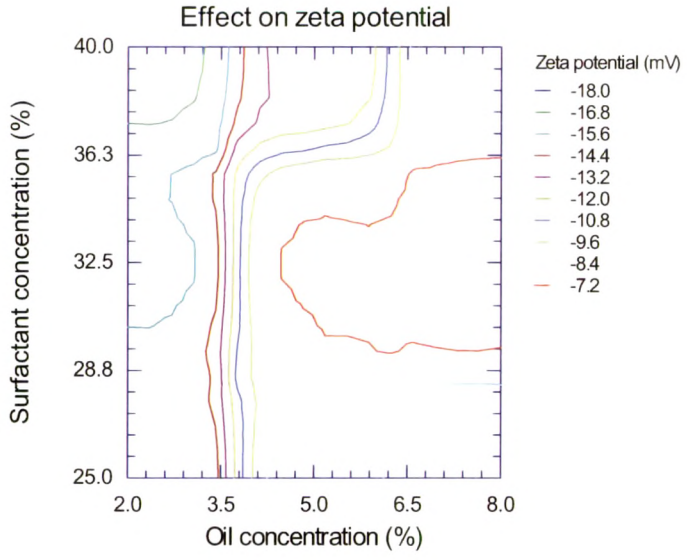


Fig 4.16: Contour plot for zeta potential of Tac system 2

$$\mathbf{Y1} = -8.14 + 4.148\,\mathbf{X1} - 0.798\,\mathbf{X2} - 2.575\,\mathbf{X11} - 1.855\,\mathbf{X22} + 0.7525\,\mathbf{X12}$$

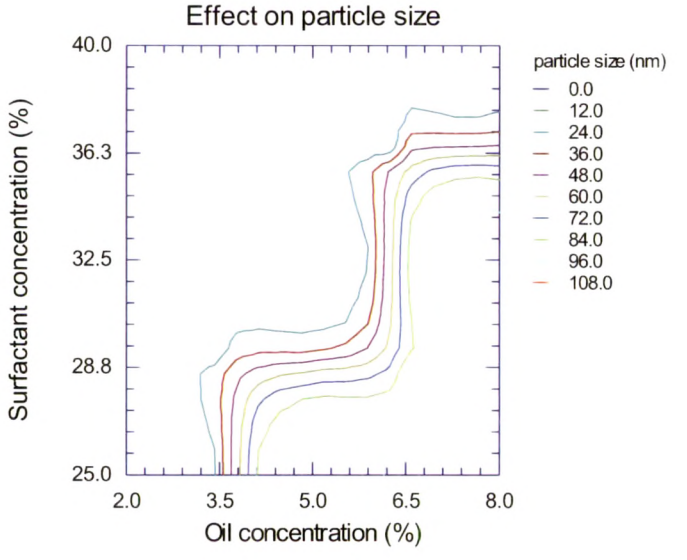


Fig 4.18: Contour plot for globule size of Tac system 2

$$\mathbf{Y2} = 41.16 + 29.697\,\mathbf{X1} - 27.342\,\mathbf{X2} + 2.57\,\mathbf{X11} + 0.235\,\mathbf{X22} - 21.145\,\mathbf{X12}$$

Table 4.14: Checkpoint batches for Tac system 2

% v/v Oil	% v/v S_{mix}	Predicted Zeta Potential (mV)	Experimental Zeta potential (mV)	Predicted Size (nm)	Experimental size (nm)
2	25	-15.6	-14.7 ± 1.7*	15.1	18.7 ± 1.9*
2	40	-16.9	-12.9 ± 2.4*	14.2	11.8 ± 3.1*
4.5	32	-7.2	-5.9 ± 0.7*	17.1	23.8 ± 2.9*
4.5	40	-13.01	-9.9 ± 1.0*	14.6	15.8 ± 2.7*
7	25	-9.13	-6.7 ± 1.3*	103.9	69.2 ± 12.3*
7	40	-7.56	-4.8 ± 1.4*	18.6	26.3 ± 4.2*

*Difference between predicted and experimental values were found to be insignificant ($P>0.05$). ** n = 3

Table 4.15: 3^2 Factorial design for Tac system 3

(Ethyl oleate, Tween 80 + Transcutol P, Distilled water) (3:1)

Formulation	% v/v Oil	% v/v S_{mix}	Zeta potential* (mV)	Globule size* (nm)	%Transmittance (630nm)	
					Before Dilution	Dilution (1 in 10)
F1	2.5	25	-9.02 ± 1.2	12.5 ± 1.7	>99%	>99%
F2	2.5	32.5	-9.2 ± 1.6	11.6 ± 1.9	>99%	>99%
F3	2.5	40	-9.7 ± 1.4	11.4 ± 1.6	>99%	>99%
F4	5	25	- 11.1 ± 2.8	84.3 ± 6.9	88 %	TOD
F5	5	32.5	-12.5 ± 1.7	81.1 ± 7.5	>99%	>99%
F6	5	40	-8.53 ± 2.8	23.9 ± 2.6	>99%	>99%
F7	7.5	25	-5.43 ± 1.9	71.7 ± 10.9	89 %	TOD
F8	7.5	32.5	-7.83 ± 1.8	124.2 ± 21.5	72 %	TOD
F9	7.5	40	-6.92 ± 1.4	42.8 ± 5.8	>99%	>99%

Optimization batches

F10	**3.5**	**30**	**-9.7 ± 1.4**	**26.3 ± 2.9**	**>99%**	**>99%**
F11	4	30	-10.9 ± 2.5	60.4 ± 8.3	92%	69%
F12	3.5	35	-10.1 ± 1.2	25.7 ± 2.7	>99%	>99%
F13	4	35	-11.8 ± 1.8	74.3 ± 7.1	>90%	TOD
F14	3.75	32	-12.3 ± 1.6	35.9 ± 4.3	>99%	>99%
F15	2	25	-10.1 ± 1.8	14.7 ± 2.1	>99%	>99%
F16	2	40	-9.5 ± 2.3	13.8 ± 3.1	>99%	>99%
F17	4.5	32	-10.9 ± 0.7	93.8 ± 12.9	79%	TOD
F18	4.5	40	-7.9 ± 1.0	20.8 ± 2.9	>99%	>99%
F19	7	25	-4.7 ± 1.9	69.4 ± 13.3	94%	73%
F20	7	40	-5.8 ± 1.9	46.3 ± 6.2	>99%	>99%

* measured for the dispersion of 1 in 10 dilution. TOD - Turbid.on dilution

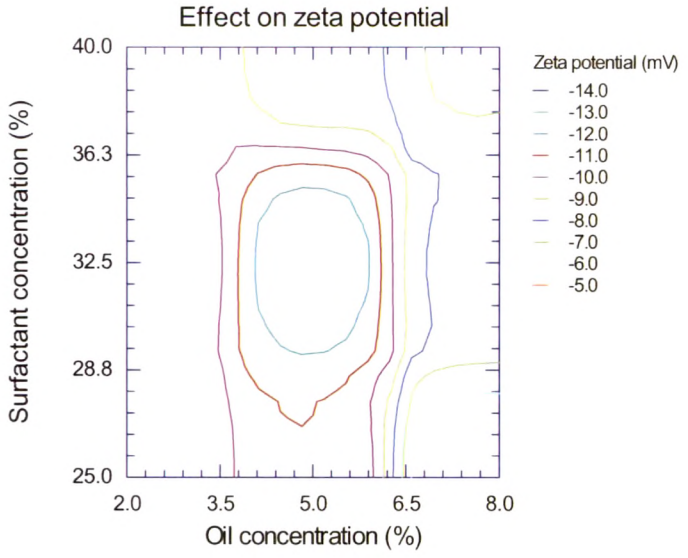


Fig 4.19 Contour plots for zeta potential of Tac system 3

Y1 = 83.914 + 33.758 **X1** – 15.178 **X2** – 17.092 **X11** – 31.872 **X22** – 6.983 **X12**

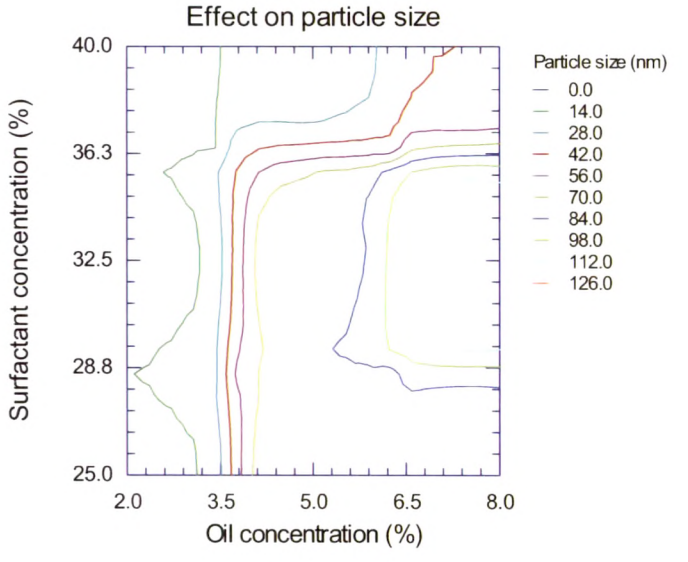


Fig 4.20 Contour plots for Globule size of Tac system 3

Y2 = -11.617 + 1.29 **X1** + 0.05 **X2** + 2.66 **X11** + 1.41 **X22** - 0.203 **X12**

Table 4.16: Checkpoint batches for Tac system 3

% v/v Oil	% v/v S_{mix}	Predicted Zeta Potential (mV)	Experimental Zeta potential (mV)	Predicted Size (nm)	Experimental size (nm)
2	25	-9.02	-10.1 ± 1.8*	12.53	14.7 ± 2.1*
2	40	-9.69	-9.5 ± 2.3*	11.41	13.8 ± 3.1*
4.5	32	-12.47	-10.9 ± 0.7*	80.56	93.8 ± 12.9*
4.5	40	-8.43	-7.9 ± 1.0*	22.95	20.8 ± 2.9*
7	25	-5.47	-4.7 ± 1.9*	71.21	69.4 ± 13.3*
7	40	-6.93	-5.8 ± 1.9*	41.96	46.3 ± 6.2*

*Difference between predicted and experimental values were found to be insignificant (P>0.05). ** n = 3

Table 4.17: Optimized Tac formulations

Test	D12	E11	F10
Zeta potential(mV)	**-10.8 ± 1.7**	-13.9 ± 2.5	-9.7 ± 1.4
Globule size(nm)	**15.9 ± 1.8**	20.4 ± 2.3	26.3 ± 2.9
%Transmittance	**>99%**	>99%	>99%
pH at 25°C	**5.6**	5.75	5.6
Drug Loading (mg/10 ml)	**48**	36	22
Assay (%)	**99.1 ± 2.6 %**	97.9 ± 1.4 %	99.5 ± 1.7 %
Viscosity at 25°C (cP)	**58.6 ± 4.42**	56.37 ± 2.85	52.3 ± 1.61

Table 4.18: Characterization of Tacrolimus Microemulsion Based Cream (TacMEC) 0.1%

Test	TacMEC 0.1%
Appearance	White smooth textured
pH at 25°C	5.2
Assay (%)	95.5 ± 1.12
Viscosity at 25°C (KcP)	43.23 ± 2.84

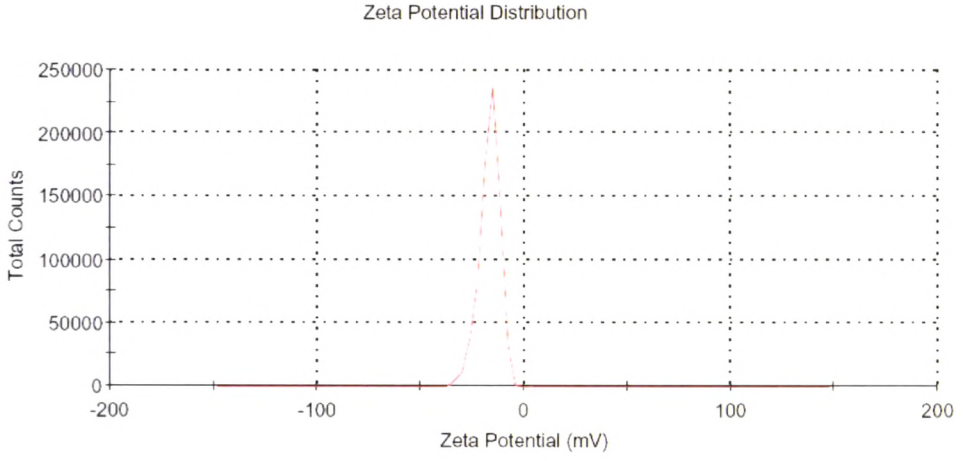


Fig. 4.21: Zeta potential distribution of the optimized batch D12

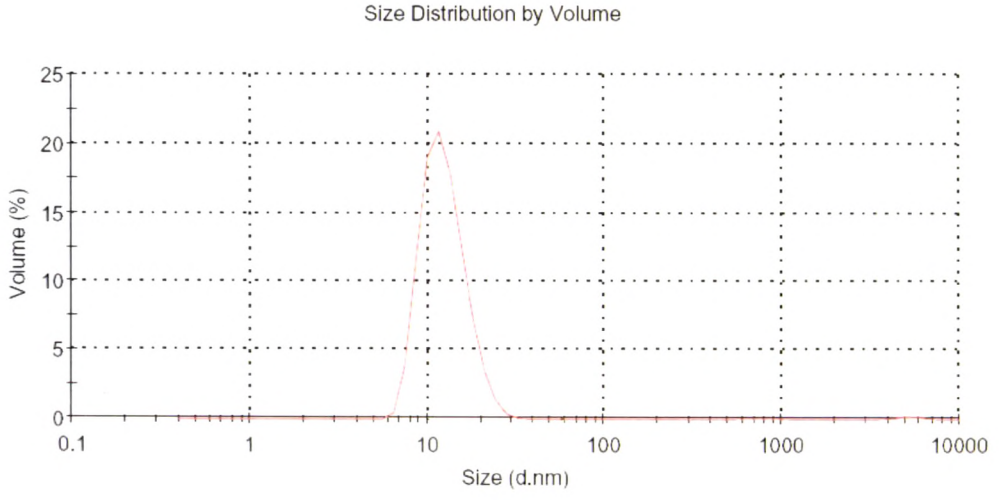


Fig. 4.22: Size distribution of the optimized batch D12

4.4.4 Transmission Electron Microscopy

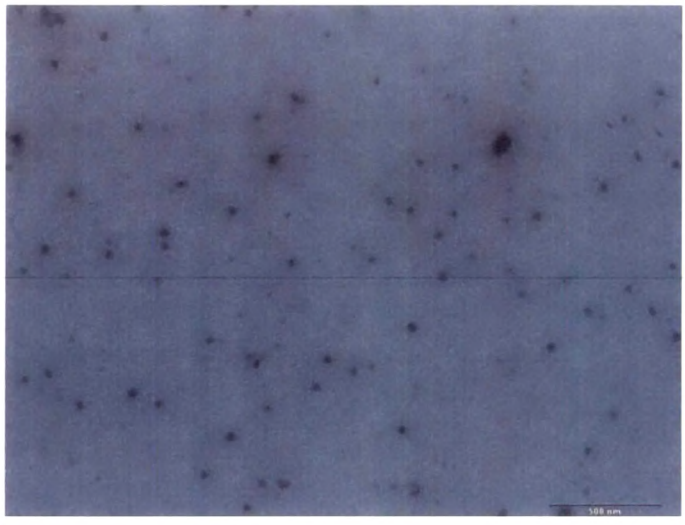


Fig 4.23: Transmission electron microscopic image of HP ME

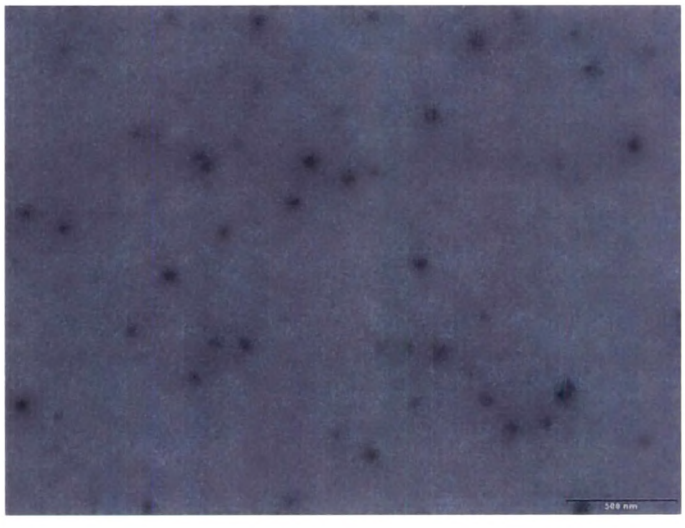


Fig 4.24 Transmission electron microscopic image of Tac ME

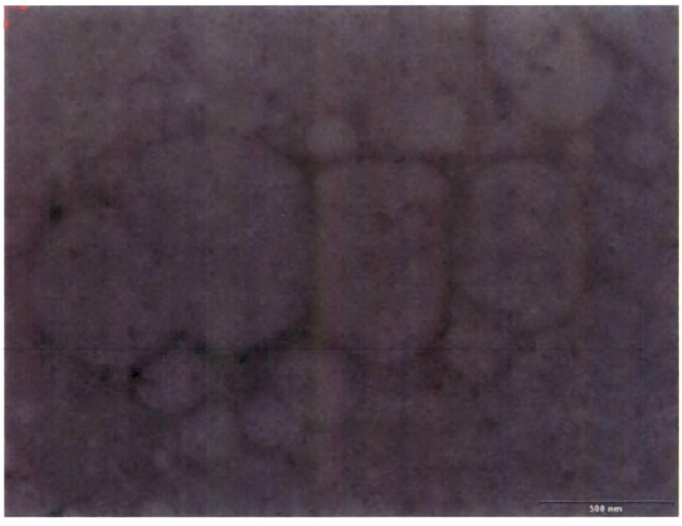


Fig 4.25: TEM image of cetomacrogol cream base after negative staining with phosphotungstic acid.

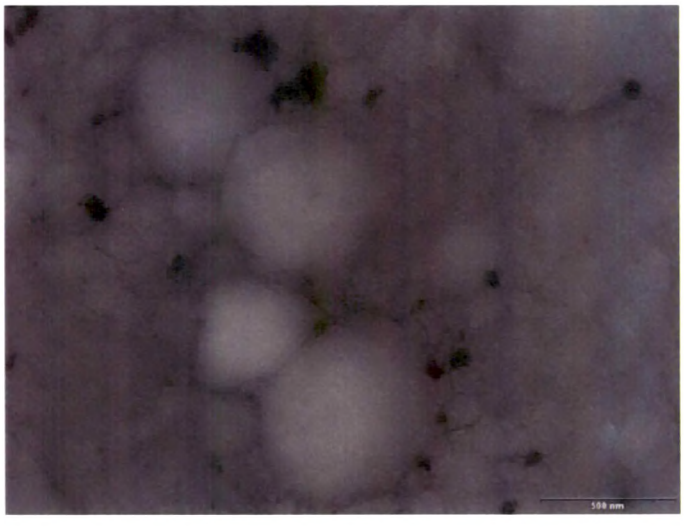


Fig 4.26: TEM image of cetomacrogol cream base after incorporation of HP ME and negative staining with phosphotungstic acid.

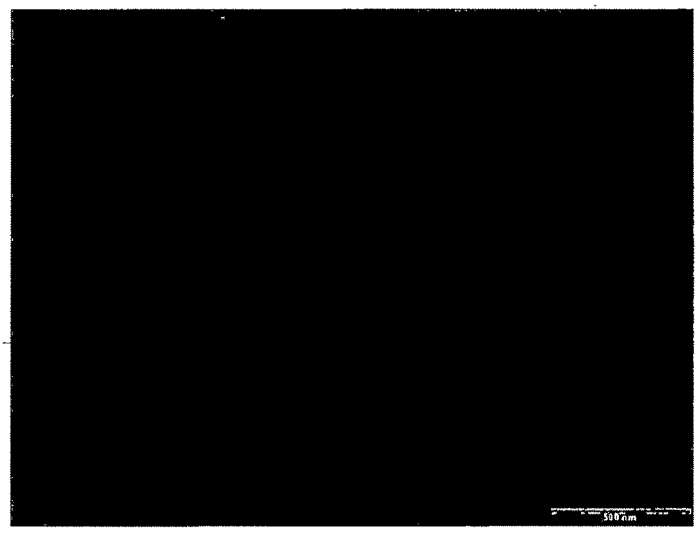


Fig 4.27: TEM image of cetomacrogol cream base after incorporation of Tac ME and negative staining with phosphotungstic acid.

4.4.5 Stability Studies

Table 4.19: Accelerated Stability Study of HP ME and Tac ME

S. No	Parameters	A14	C12	D12	E11
Before ASS					
1.	ZP	-10.8 ± 1.9	-9.6 ± 1.8	-10.8 ± 1.9	-13.9 ± 2.5
2.	GS	14.8 ± 1.1	18.6 ± 2.7	15.9 ± 1.8	20.4 ± 2.3
3	%T	>99%	>99%	>99%	>99%
After Centrifugation					
4.	ZP	-11.3 ± 2.6	-9.9 ± 2.3	-11.6 ± 2.8	-10.9 ± 1.9
5.	GS	18.9 ± 2.3	21.2 ± 3.5	22.3 ± 3.4	22.5 ± 1.9
6.	%T	>99%	>99%	>99%	>99%
After Freeze thaw cycle					
7.	ZP	-12.2 ± 2.1	-7.9 ± 2.5	-12.2 ± 3.1	-11.3 ± 1.5
8.	GS	16.4 ± 1.5	17.6 ± 4.1	17.9 ± 2.1	21.1 ± 1.1
9.	%T	>99%	>99%	>99%	>99%
After Heating cooling cycle					
10.	ZP	-8.9 ± 2.4	-9.1 ± 2.1	-10.9 ± 2.2	-12.4 ± 2.4
11.	GS	15.6 ± 2.7	19.9 ± 3.7	20.1 ± 2.4	23.1 ± 3.1
12.	%T	>99%	>99%	>99%	>99%

(ZP- zeta potential; GS - globule size; %T - % transmittance)

Table 4.20: Stability study of HP MEs

System	Period (month)	Room temperature				2-8° C			
		Zeta potential (mV)*	Globule size(nm)*	% trans mittance	% drug content	Zeta potential (mV)*	Globule size(nm)*	% trans mittance	% drug content
A14	0	-10.8 ± 1.9	14.8 ± 1.1	>99%	99.6 ± 1.3	-10.8 ± 1.9	14.8 ± 1.1	>99%	99.6 ± 1.3
	1	-9.9 ± 2.1	18.2 ± 1.8	>99%	98.9 ± 1.9	-10.4 ± 2.3	15.7 ± 1.5	>99%	99.2 ± 2.1
	2	-11.2 ± 1.7	16.8 ± 2.1	>99%	98.6 ± 1.6	-11.1 ± 2.9	17.8 ± 1.9	>99%	99.0 ± 1.8
	3	-8.6 ± 2.5	21.3 ± 1.9	>99%	97.1 ± 2.3	-9.8 ± 3.9	18.8 ± 1.1	>99%	98.9 ± 3.1
	6	-9.5 ± 1.3	23.4 ± 3.1	>99%	97.2 ± 2.8	-9.8 ± 2.7	17.9 ± 3.1	>99%	99.0 ± 1.8
C12	0	-9.6 ± 1.8	18.6 ± 2.7	>99%	99.1 ± 1.9	-9.6 ± 1.8	18.6 ± 2.7	>99%	99.1 ± 1.9
	1	-9.2 ± 1.3	21.5 ± 2.5	>99%	97.9 ± 1.2	-9.8 ± 1.2	19.2. ± 2.7	>99%	98.5 ± 1.5
	2	-8.5 ± 2.5	22.6 ± 1.7	>99%	98.2 ± 1.7	-8.8 ± 1.6	20.5 ± 2.9	>99%	97.6 ± 2.1
	3	-8.3 ± 3.1	24.2 ± 3.5	>99%	97.6 ± 2.8	-8.9 ± 2.5	21.6 ± 2.3	>99%	97.5 ± 1.9
	6	-8.2 ± 2.1	26.7 ± 4.7	>99%	97.2 ± 3.1	-8.9 ± 3.8	26.8 ± 4.5	>99%	97.1 ± 3.6

* Zeta potential and globule size were measured for the dispersion of 1 in 10 dilution in distilled water at 25ºC

Table 4.21: Stability study of Tac MEs

System	Period (month)	Room temperature				2-8° C			
		Zeta potential (mV)*	Globule size(nm)*	% trans mittance	% drug content	Zeta potential (mV)*	Globule size(nm)*	% trans mittance	% drug content
D12	0	-10.8 ± 1.7	15.9 ± 1.8	>99%	99.1 ± 2.6	-10.8 ± 1.7	15.9 ± 1.8	>99%	99.1 ± 2.6
	1	-11.4 ± 1.4	19.3 ± 1.4	>99%	99.0 ± 3.5	-11.6 ± 1.6	19.8 ± 1.1	>99%	98.8 ± 2.8
	2	-11.2 ± 1.3	19.9 ± 1.9	>99%	98.5 ± 2.9	-11.9 ± 1.5	20.9 ± 1.3	>99%	98.6 ± 2.5
	3	-11.6 ± 2.9	21.5 ± 2.1	>99%	98.1 ± 3.6	-11.9 ± 2.9	20.9 ± 1.9	>99%	98.4 ± 2.6
	6	-10.9 ± 2.5	22.6 ± 3.7	>99%	97.5 ± 4.4	-11.6 ± 2.5	21.5 ± 1.4	>99%	97.3 ± 3.9
E11	0	-13.9 ± 2.5	20.4 ± 2.3	>99%	97.9 ± 1.4	-13.9 ± 2.5	20.4 ± 2.3	>99%	97.9 ± 1.4
	1	-13.5 ± 2.9	24.1 ± 3.3	>99%	97.1 ± 2.7	-13.7 ± 3.1	22.4 ± 2.5	>99%	96.9 ± 2.4
	2	-12.9 ± 3.5	23.9 ± 2.6	>99%	96.5 ± 2.5	-13.4 ± 2.2	23.2 ± 2.6	>99%	97.1 ± 1.9
	3	-12.6 ± 4.2	26.1 ± 3.8	>99%	96.4 ± 1.9	-12.9 ± 3.5	23.1 ± 3.3	>99%	97.2 ± 1.8
	6	-12.2 ± 2.7	29.4 ± 3.9	>99%	95.8 ± 1.2	-12.1 ± 1.5	24.6 ± 2.9	>99%	96.4 ± 3.4

* Zeta potential and globule size were measured for the dispersion of 1 in 10 dilution in distilled water at 25ºC

Table 4.22: Stability study of HPMEC 0.035%, HPMEC 0.05% and TacMEC 0.1%

System	Period (month)	Room temperature				2-8° C			
		Appearance	pH	Assay (%)	Viscosity (KcP)	Appearance	pH	Assay (%)	Viscosity (KcP)
HPMEC 0.035%	0	White smooth textured	5.4	96.7 ± 1.6	38.9 ± 2.3	White smooth textured	5.4	96.7 ± 1.6	38.9 ± 2.3
	1	Same	5.4	96.3 ± 2.1	39.3 ± 2.4	Same	5.4	96.7 ± 2.4	38.9 ± 2.5
	2	Same	5.3	96.1 ± 1.9	38.1 ± 1.9	Same	5.4	96.1 ± 2.1	39.2 ± 1.9
	3	Same	5.3	95.6 ± 1.8	37.2 ± 1.7	Same	5.3	95.4 ± 1.9	38.1 ± 1.7
	6	Same	5.3	95.0 ± 2.4	36.2 ± 2.4	Same	5.3	95.6 ± 2.8	38.0 ± 3.0
HPMEC 0.05%	0	White smooth textured	5.3	97.2 ± 2.3	42.1 ± 3.1	White smooth textured	5.3	97.2 ± 2.3	42.1 ± 3.1
	1	Same	5.3	97.5 ± 2.1	41.0 ± 3.0	Same	5.3	97.0 ± 3.1	41.6 ± 2.3
	2	Same	5.2	97.1 ± 2.9	41.3 ± 2.4	Same	5.2	96.5 ± 4.1	41.3 ± 2.7
	3	Same	5.1	97.2 ± 1.6	40.6 ± 2.6	Same	5.2	96.7 ± 2.6	41.2 ± 2.8
	6	Same	5.1	96.1 ± 3.4	40.8 ± 1.9	Same	5.2	96.6 ± 1.5	41.7 ± 3.2
TacMEC 0.1%	0	White smooth textured	5.2	95.5 ± 1.1	43.2 ± 2.8	White smooth textured	5.2	95.5 ± 1.1	43.2 ± 2.8
	1	Same	5.1	95.2 ± 2.8	43.0 ± 1.7	Same	5.1	95.2 ± 2.5	43.1 ± 2.2
	2	Same	5.1	95.1 ± 1.8	42.1 ± 2.6	Same	5.1	95.1 ± 2.4	42.6 ± 2.4
	3	Same	5.1	94.3 ± 1.9	42.1 ± 3.4	Same	5.1	95.3 ± 1.7	42.5 ± 2.9
	6	Same	5.1	94.1 ± 3.2	41.4 ± 2.1	Same	5.1	94.2 ± 3.3	42.1 ± 3.4

4.5 Discussion

4.5.1 Halobetasol propionate microemulsion and microemulsion based cream

Microemulsions of halobetasol propionate were successfully prepared by construction of pseudo ternary phase diagram using titration method. Based on the solubility study data shown in table 4.1, Capmul MCM L8 and isopropyl myristate was selected as an internal phase for the preparation of microemulsion. Isopropyl myristate was explored because the marketed cream and ointments have isopropyl myristate as one of the excipients. Further, isopropyl myristate has also been reported as a penetration enhancer. The selection of surfactant and cosurfactant mixture was on the basis of HLB values, drug solubility, safety and stability profile. Non-ionic surfactants are known to be least toxic and chemically highly stable and hence, use of non-ionic surfactant for pharmaceutical microemulsion formulation is gradually increasing. Surfactant, Tween 80 was selected for the study along with cosurfactants like Transcutol P and PEG 200.

Different ratios of surfactant and cosurfactant (1:1 to 3:1) were studied in the phase diagram construction. The phase study revealed that increasing the S_{mix} ratio from 1:1 to 3:1, the microemulsion region increased toward water-oil axis. (Fig. 4.1, 4.2, 4.3). This indicates that increasing surfactant mix concentration, the maximum amount of oil can be solubilised/ emulsified. This was earlier reported by Lianli et al (2002) and Zhang et al (2004). The increased oil content may provide opportunity for the solubilisation of the drug. However, the final ratio of surfactant: co-surfactant was selected keeping in view that solubility of drug is higher in co-surfactant and increased co-surfactant may provide an opportunity for higher drug loading. For halobetasol propionate 3 systems were prepared which are System1

[Capmul MCM L8, Tween 80 + Transcutol P (1:1), Distilled water] and System2 [Isopropyl Myristate, Tween 80 + Transcutol P (2:1), Distilled water] and system 3 (Capmul MCM L8, {Tween 80 + (Transcutol P: PEG 200(1:1)) (2:1)}, Distilled water].

Experimental design (3^2) (Table 4.3, 4.5 & 4.7) was utilized in the formulation of microemulsion by varying oil content from 2.5%v/v to 7.5%v/v and S_{mix} from 25%v/v to 40%v/v, measuring globule size (GS) and zeta potential (ZP) as responses. It was found that low surfactant content / high oil content resulted microemulsions with large size. It was obvious that the zeta potential was contributed by both the contents of dispersed phase and S_{mix} content (Malmsten Martin 2002).

It was observed that the zeta potential and globule size of microemulsions were influenced by the dilution which was made before measurement. Since the low interparticle space between the globules results in multiple light scattering which leads to a false measurement. The dilution and the temperature at which the measurements have to be made were kept constant for through out the study for all the systems. The zeta potential and globule size were measured for the microemulsions of 1 in 10 dilution in distilled water at 25ºC.

The combined effect of oil content and S_{mix} concentration on zeta potential and globule size were illustrated by contour plots (Fig 4.4 - Fig. 4.9). The change in the zeta potential and globule size with the change in both oil and S_{mix} content was found to follow a systematic pattern. Check point experiments (Table 4.4, 4.6, 4.8) were performed to confirm the utility of polynomial equation and established contour plots in the preparation of microemulsion. In all the 3 systems, it was found that the globule sizes were below 50nm. The optimized

batches of system1 system2, and system 3 were selected on the basis of highest zeta potential value with the globules size less than 30nm (Table 4.9) and batch A14 was selected for further studies and incorporation into cream base.

The selected microemulsions were characterized for drug loading, qualitative test, zeta potential, globule size, %transmittance, pH, assay and viscosity and the results were recoded in Table 4.9. When the microemulsion systems were diluted with water, it was readily miscible with water. When methyl orange was added to the microemulsions, they resulted into colored solutions without any clumps. These both dilution test and dye tests indicated that the prepared microemulsions are of o/w type microemulsion. The pHs of the formulations were found to be within the range of skin surface pH and hence would not cause irritation. Microemulsions were found to possess lower viscosity and exhibit newtonian flow. The batch A14 was selected on the basis of drug loading, particle size and zeta potential for further development.

In the TEM positive image of HPME, microemulsion appeared dark and the surroundings were bright (Fig 4.23). The size of oil globules were in agreement with the globule size distribution measured using photon correlation spectroscopy.

Cetomacrogol cream base was prepared according to the formula described in British Pharmaceutical Codex. The drug loaded microemulsion was incorporated in cetomacrogol cream base by replacing an equivalent quantity of water so as to give 0.035% and 0.05% concentration of halobetasol in the final formulation. 0.05% is the clinical concentration and 0.035% is a lower dose which was explored. The microemulsion is mixed at temp not above 30°C with gentle mixing. It is assumed that the microemulsion's

microstructure is not altered when incorporated in cream base. The evidence for the same was transmission electron microscopic images of cream before and after incorporation of microemulsion into it (Fig. 4.25 and 4.26). The images showed an increased number of oil globules in the size range below 50 nm. It can be assumed that since microemulsion is mixed at a point when the cream is already in semi-solid state, the microstructure of microemulsion is not disturbed significantly. Similar incorporation of microemulsion in hydrogel matrix has been reported to retain its microstructure. The characterization of HPMEC 0.035% and HPMEC 0.05% are recorded in table 4.10.

In long term stability study, the HP microemulsions (A14 & C12) were packed in the borosil screw capped vials and were kept at room temperature (25-35°C) and refrigeration temperature (2-8°C). During the storage period, microemulsion systems were assessed for their zeta potential, globule size, physical stability, assay and pH (Table 4.20). Over the time period of 6 months, there was a change in the zeta potential with an increment in globule size. But the increment in the zeta potential and globules size were found to be insignificant when no visual indications of physical instability of the systems were seen. Irrespective of the storage conditions, the systems remained stable for 6 months duration.

In order to assess the thermodynamic stability, the accelerated stability studies were done by subjecting the formulations for centrifugation, freeze-thaw cycle and heating cooling cycle. The A14 and C12 were centrifuged; freeze thawed and kept them at 45°C and 4°C alternatively. Before and after each treatment, zeta potential, globule size and %transmittance of the formulations were determined and recorded (Table 4.19). The change in the parameters after accelerated stability conditions was found to be insignificant

which clearly indicates that the prepared microemulsion systems were thermodynamically stable. The HP MEC 0.035% and HPMEC 0.05%b was also found to be stable for a period of 6 months (Table 4.22).

4.5.2 Tacrolimus microemulsion and microemulsion based cream

Microemulsions of tacrolimus were successfully prepared by construction of pseudo ternary phase diagram using titration method. Based on the solubility study data shown in Table 4.2, Capmul MCM C8 and ethyl oleate were selected as an internal phase for the preparation of microemulsion. The selection of surfactant and cosurfactant mixture was on the basis of HLB values, drug solubility, safety and stability profile. Non-ionic surfactants are known to be least toxic and chemically highly stable and hence, use of non-ionic surfactant for pharmaceutical microemulsion formulation is gradually increasing. Surfactant, Tween 80 was selected for the study along with cosurfactants like Transcutol P and Soluphor P.

Different ratios of surfactant and cosurfactant (0.5:1 to 3:1) were studied in the phase diagram construction. The phase study revealed that increasing the S_{mix} ratio, the microemulsion region increased toward water-oil axis. (Fig. 4.12, 4.13 and 4.14). This indicates that increasing surfactant mix concentration, the maximum amount of oil can be solubilised/ emulsified. This was earlier reported by Lianli et al (2002) and Zhang et al (2004). The increased oil content may provide opportunity for the solubilisation of the drug. However, the final ratio of surfactant: co-surfactant was selected keeping in view that solubility of drug is higher in co-surfactant and increased co-surfactant may provide an opportunity for higher drug loading. For tacrolimus 3 systems were prepared which are System1 [Capmul MCM C8, Tween 80 + Transcutol P (1:1), Distilled water] and System2 [Capmul MCM C8, Tween 80 + Soluphor

P (1:1), Distilled water] and system 3 (Ethyl oleate, Tween 80 + Transcutol P (2:1), Distilled water].

Experimental design (3^2) (Table 4.11, 4.13 & 4.15) was utilized in the formulation of microemulsion by varying oil content from 2.5%v/v to 7.5%v/v and S_{mix} from 25%v/v to 40%v/v, measuring globule size (GS) and zeta potential (ZP) as responses. It was found that low surfactant content / high oil content resulted microemulsions with large size. It was obvious that the zeta potential was contributed by both the contents of dispersed phase and S_{mix} content (Malmsten Martin 2002).

It was observed that the zeta potential and globule size of microemulsions were influenced by the dilution which was made before measurement. Since the low interparticle space between the globules results in multiple light scattering which leads to a false measurement. The dilution and the temperature at which the measurements have to be made were kept constant for through out the study for all the systems. The zeta potential and globule size were measured for the microemulsions of 1 in 10 dilution in distilled water at 25ºC.

The combined effect of oil content and S_{mix} concentration on zeta potential and globule size were illustrated by contour plots (Fig 4.15 - Fig. 4.20). The change in the zeta potential and globule size with the change in both oil and S_{mix} content was found to follow a systematic pattern. Check point experiments (Table 4.12, 4.14, 4.16) were performed to confirm the utility of polynomial equation and established contour plots in the preparation of microemulsion. In all the 3 systems, it was found that the globule sizes were below 50nm. The optimized batches of system1 system2, and system 3 were selected on the

basis of highest zeta potential value with the globules size less than 30nm (Table 4.16).

The selected microemulsions were characterized for drug loading, qualitative test, zeta potential, globule size, %transmittance, pH, assay and viscosity and the results were recoded in Table 4.16. When the microemulsion systems were diluted with water, it was readily miscible with water. When methyl orange was added to the microemulsions, they resulted into colored solutions without any clumps. These both dilution test and dye tests indicated that the prepared microemulsions are of o/w type microemulsion. The pH of the formulations were found to be within the range of skin surface pH and hence would not cause irritation. Microemulsions were found to possess lower viscosity and exhibit newtonian flow. The batch D12 was selected on the basis of drug loading, particle size and zeta potential for further development.

In the TEM positive image of tacrolimus loaded microemulsion, microemulsion appeared dark and the surroundings were bright (Fig 4.24). The size of oil globules were in agreement with the globule size distribution measured using photon correlation spectroscopy.

Cetomacrogol cream base was prepared according to the formula described in British Pharmaceutical Codex. The drug loaded microemulsion was incorporated in cetomacrogol cream base by replacing an equivalent quantity of water so as to give 0.1% concentration of tacrolimus in the final formulation. The microemulsion is mixed at temp not above 30°C with gentle mixing. It is assumed that the microemulsion's microstructure is not altered when incorporated in cream base. The evidence for the same was transmission electron microscopic images of cream before and after incorporation of microemulsion into it (Fig. 4.21 and 4.23). The images

showed an increased number of oil globules in the size range below 50 nm. It can be assumed that since microemulsion is mixed at a point when the cream is already in semi-solid state, the microstructure of microemulsion is not disturbed significantly. Similar incorporation of microemulsion in hydrogel matrix has been reported to retain its microstructure. The characterization of TacMEC 0.1% is recorded in table 4.18.

In long term stability study, the TC microemulsions (D12 & E11) were packed in the borosil screw capped vials and were kept at room temperature (25-35°C) and refrigeration temperature (2-8°C). During the storage period, micro emulsion systems were assessed for their zeta potential, globule size, physical stability, assay and pH (Table 4.21). Over the time period of 6 months, there was a change in the zeta potential with an increment in globule size. But the increment in the zeta potential and globules size were found to be insignificant when no visual indications of physical instability of the systems were seen. Irrespective of the storage conditions, the systems remained stable for 6 months duration.

In order to assess the thermodynamic stability, the accelerated stability studies were done by subjecting the formulations for centrifugation, freeze-thaw cycle and heating cooling cycle. The D12 and E11 were centrifuged; freeze thawed and kept them at 45°C and 4°C alternatively. Before and after each treatment, zeta potential, globule size and %transmittance of the formulations were determined and recorded (Table 4.19). The change in the parameters after accelerated stability conditions was found to be insignificant which clearly indicates that the prepared microemulsion systems were thermodynamically stable. The TacMEC 0.1% was also found to be stable for a period of 6 months (Table 4.22).

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