Chapter 4

Formulation & Characterization

4.1 INTRODUCTION

Microemulsions are thermodynamically stable systems that are broadly categorized into three types. 1. oil-in-water (o/w) microemulsions 2. water-in-oil (w/o) microemulsions 3. Bicontinuous microemulsions. Many researchers in various literatures have reported the formulation techniques for microemulsion. These techniques are mainly pseudo ternary diagram construction and titration method (Lawrence and Rees 2000). Regardless of the type of microemulsion systems, microemulsions 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 microemulsions 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 microemulsion (ME) system. Ternary phase diagram can be represented in a triangular format, in which each coordinate represents one component of microemulsion 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 1. Rapid 2. Reasonably accurate and precise 3. 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 bicontinues 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

- Clobazam and Clopidogrel bisulphate were gifted by Sun Pharma Advanced Research Company Ltd., Vadodara, Gujarat
- Captex 100, Capmul MCM, Caprol 3GO, Caprol 10G-100, Capmul GMO – 50EP/NF, Lauroglycol 90, Lauroglycol FCC, Captex 355 EP/NF, Caprol ET, Capmul MCM C10, Acconan CC6, Captex 8000, Capmul MCM C8, Captex

500, Capryol PGMC were gifted by Abitec Corporation Limited, Janesville, USA.

- Labrafac PG, Labrafac Lipophile WL 1349, Peceol, Capryol 90, Labrafil M1944 Cs, Transcutol, Labrafac CC, Labrafac CC, Labrafil M 2125 were gifted by Gattefosseee, 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.
- Chitosan was gifted by Indian sea Foods Limited, Kochin. India.
- Polycarbophil 940P was purchased from Himedia laboratories limited, Mumbai. India.
- HPMC K100LV was purchased from Colorcon Asia private Limited, Goa. India.
- Other chemicals were of analytical grade and purchase from Sd fine chemicals, Mumbai.

Instruments:

The instruments used for the preparation and estimation of drugs in the formulation include UV-Visible Spectrophotometer, (UV 1700,Shimadzu, Japan), pH meter (Labindia), Bath sonicator, analytical balance, magnetic stirrer (Remi, Viscometer (Brookfeild HADV), Sigma centrifuge, Malvern instruments, Abdobe Refractometer, Transmission Emission Microscope.

Glassware:

Pipettes of 1 ml and 10 ml capacity, volumetric flasks of 10 ml capacity, beaker and other glassware of Borosil

4.3 SOLUBILITY DETERMINATION

Solubility of drugs clobazam and clopidogrel bisulphate was determined in different oils, surfactants and cosurfactans. Drugs were added in excess to different oils, surfactants and cosurfactants and shaken by mechanical shaker for 24 hours. The samples were centrifuged at 8000 rpm for 10 minutes and the drug content in the supernant 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 clobazam and clopidogrel bisulphate respectively.

4.4 PREPARATION OF MUCOADHESIVE MICROEMULSION

4.4.1 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 vigorous stirring. After equilibrium, the samples were visually checked and determined as being clear microemulsions. No heating was conducted during the preparation. Phase diagrams were constructed using Chemix software. The phase diagram with different ratios of surfactant and cosurfactant with different oils were constructed to explore the microemulsion 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, oils (Capmul MCM, Capmul GMO, oleic acid) surfactant (Tween 20, Tween 80, Acconan CC6) and cosurfactant (Transcutol, ethanol, PEG 200) were selected and different ratios of surfactant and cosurfactant (1:1 to 4:1) were studied in the phase diagram construction. Different systems were studied and listed below.

- Labrafil, Tween 20:PEG200, Distilled water.
- Capmul MCM, Labrasol:Transcutol P, Distilled water.
- Oleic acid, Labrasol:Transcutol P, Distilled water.
- Capmul GMO,Tween 20:Ethanol, Distilled water.
- Capmul MCM C-10, Tween 20: Ethanol, Distilled water.
- Capmul MCM, Acconan CC6: Tween 20, Distilled Water.
- Capmul MCM, Acconan CC6: Tween 80, Distilled Water.
- Capmul MCM, Acconan CC6: Ethanol, Distilled Water.
- Capmul MCM, Tween 20: Transcutol P, Distilled Water.
- Capmul GMO, Tween 20: Transcutol P, Distilled water.
- Capmul GMO, Tween 80: Transcutol P, Distilled water.
- Capmul GMO, Tween 20: Transcutol P, Acetate Buffer (pH 5).
- Capmul GMO, Tween 20: PEG 200, Acetate Buffer (pH 5).

4.4.2 Preparation of Microemulsion:

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

Clobazam microemulsion (CZME):

i System 1: Capmul MCM, Acconan CC6: Tween 20 (3:1), Distilled Water.

ii System 2: Capmul MCM, Tween 20: Transcutol P (3:1), Distilled Water

Two microemulsion systems containing clobazam were explored and the results were recorded in Table 4.3 & Table 4.8.

Clopidogrel bisulphate microemulsion (CSME):

i System 1: Capmul GMO, Tween 80: Transcutol P (2:1), Distilled water.

ii System 2: Capmul GMO, Tween 20: Transcutol P (2:1), Acetate buffer (pH 5).

iii System 3: Capmul GMO, Tween 20: PEG 200 (3:1), Acetate buffer (pH 5)

Three microemulsion systems containing clopidogrel bisulphate were explored and the results were recorded in Table 4.14, 4.16 & 4.20

4.4.3 Optimisation of microemulsion preparation:

Experimental design (3^2) was applied in the formulation of microemulsion by varying concentrations/ levels of oil and S_{mix} and measuring globule size (GS) and zeta potential (ZP) as the responses. Nine batches of microemulsions 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 clobazam microemulsion systems 1 and 2 were shown in the Tables 4.4 & Table 4.9 respectively. Similarly the factorial design of clopidogrel bisulphate microemulsion systems 1, 2 and 3 were shown in the Tables 4.15, 4.17 & Table 4.21 respectively

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

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

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 and materials in GRAS list.

A check point experiment was performed to confirm the utility of polynomial equation and established contour plots in the preparation of microemulsion. Three 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. Microemulsions 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 clobazam containing formulations were prepared and recorded in Table 4.6 & 4.10. Similarly the check point batches of clopidogrel bisulphate containing formulations were prepared and recorded in Table 4.18 & 4.22.

4.4.4 Preparation of Mucoadhesive Microemulsion:

The mucoadhesive microemulsions were prepared by first preparing a microemulsion of the drug using minimum volume of external phase and then adding the required volume of concentrated polymer solution to it such that the required final concentration of the polymer in the mucoadhesive microemulsion was obtained. 1% w/v carbopol solution was prepared by dispersing carbopol in distilled water followed by neutratlisation with 0.1N NaOH. The 2 %w/v chitosan solution was prepared by dispersing in water and allowed to hydrate for 12 hours. After the addition of the mucoadhesive solution to the microemulsion, the system was stirred for 10 minutes to homogenize. The mucoadhesive microemulsions containing clobazam were prepared and characterized and recorded in Table 4.7 and 4.11. Similarly mucoadhesive microemulsions containing clopidogrel bisulphate were prepared and characterized and recorded in Table 4.19 and 4.23.

4.4.5 Preparation of drug solutions:

Clobazam solution (CZS, 3mg/ml) was prepared by dissolving clobazam in a mixture of propylene glycol, PEG 200, ethanol, Tween 20 (60%,20%,12%, 8% v/v).

Clopidogrel bisulphate solution (CSS, 4mg/ml) was prepared by dissolving clopidogrel bisulphate in a mixture of propylene glycol and water (3:1 ratio).

4.5 CHARACTERISTAION

4.5.1 Qualitative tests:

These tests were used to determine the type of microemulsion.

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 microemulsion preparation. Hence, in case of o/w system the microemulsion can be diluted with the aqueous phase while with w/o microemulsion the system is diluted with the oil used.

Dye solubility test: It is also known as the ***staining test.*** Staining tests in which a dye is sprinkled on the surface of the emulsion also indicate the nature of the continuous phase. With an o/w emulsion there is rapid dispersion of a water-soluble dye into the system where as with w/o emulsion the dye forms microscopically visible clumps. The reverse happens on addition of an oil-soluble dye. These tests essentially identify the continuous phase.

4.5.2 Globule size determination:

The globule size determination (Kaler and Prager 1982; Roland et al 2003) of CZME and CSME 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 (PCS) 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 microemulsion 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. Effect of dilution on zeta potential and globule size was studied and shown for CZ system1 in Table 4.5.

4.5.3 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 microemulsion. 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. Latex dispersion having zeta potential –50 mV ± 2.5 mV was used as a standard.

4.5.4 Transmittance: The % transmittance of the microemulsion was checked against distilled water using UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan) at **630nm.**

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

4.5.6 Assay: Assay of the microemulsions were determined as per the methods described in the Analytical section and the results were recorded in Tables 4.12 & 4.24.

4.5.7 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 33.8±0.3°C and the results were recorded in Tables 4.12 & 4.24.

4.5.8 Transmission Electron microscopy(TEM)

TEM is used as a tool to study the morphology and structure of the delivery systems. The TEM images of microemulsions were taken to get idea about the size of microemulsions (Sheikh Shafiq et al 2007). The images were taken Tecnai200 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) microemulsion was directly deposited on the copper grid and observed after drying and the positive image were shown in Fig 4.15 & Fig 4.16.

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

4.6 NASAL TOXICITY STUDY

Freshly excised sheep nasal mucosa, except for the septum part was collected from the slaughter house in PBS pH 6.4. The membrane was kept in PBS pH 6.4 for 15 min. Sheep nasal mucosa pieces with uniform thickness were mounted on Franz diffusion cells. One mucosa was treated with 0.5 ml of PBS pH 6.4; the other mucosa with 0.5 ml of isopropyl alcohol and the remaining with microemulsions and mucoadhesive microemulsions for 1 hr. After 1 hr the mucosa were rinsed with PBS pH 6.4 and carried to the pathological laboratory in 10% formalin for the preparation of pathological slides. The sheep nasal mucosa treated with PBS pH 6.4 and isopropyl alcohol were taken as positive and negative control respectively. The prepared pathological slides were studied under optical microscope for any sign of toxicity and the images were stored in the form of photographs and shown in Fig 4.17(A-N).

4.7 STABILITY STUDY

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

4.7.1 Long term stability study:

In long term stability study, the microemulsions were packed in the borosil screw capped vials and were kept at room temperature (25-35°C) and refrigeration temperature (20°C). Over the time period micro emulsion systems were assessed for their zeta potential, globule size, physical stability, assay and pH. .

1) ***Zeta potential measurement:*** Zeta potential of the microemulsions were determined at predetermined time interval as described in section 4.5.3 and the results recorded in Tables 4.25 & 4.26
2) ***Globule size determinationt:*** Particle size of the microemulsions were determined at predetermined time interval as described in section 4.5.2 and recorded in Tables 4.25 & 4.26
3) ***Physical stability:*** During the storage period, the microemulsions 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 microemulsion system and recorded in Tables 4.25 & 4.26

4) ***% Assay:*** The drug content of the formulations were determined as per the method described in analytical section 3.4.1 & 3.4.2 and recorded in Tables 4.25 & 4.26

5) ***pH:*** pH of ME formulations were monitored during the storage period and recorded in Tables 4.25 & 4.26

4.7.2 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)

1. The formulations were centrifuged for 30 minute at 10,000 rpm and observed for phase separation.
2. The systems were kept for freeze/ thaw cycles between - 21°C and 25°C for not less than 48 hours at each stage for three cycles.
3. The systems were subjected to 6 cycles of heating / cooling cycle by keeping them at 4°C and 45°C 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.

TEM, refractive index, nasal toxicity study and accelerated stability studies were performed for selective formulations only.

4.8 PREPARATION AND CHARACTERIZATION OF MUCOADHESIVE GEL

Mucoadhesive gel was prepared by simple addition of polymers, hydration followed by pH adjustment (D'Souza et al 2006). The HPMC based gel was prepared as the delivery system for insulin like growth factor1. The gel consisted of HPMC K100LV (0.33% w/w) and Carbopol 940P (0.17% w/w). Required quantities of polymers were sifted, weighed and blended thoroughly. The polymers were then suspended in distilled water and allowed to swell. Triethanolamine was added drop wise with gentle stirring till a clear, smooth, and translucent gel was obtained at a pH of 5.5 to 6. Sodium metabisulphite (0.01%) and methyl paraben (0.05%) were finally incorporated and mixed well. Finally insulin like growth factor-1 which was reconstituted with phosphate buffer saline pH 6.4 was added and mixed well to obtain a uniform gel.

The gel was evaluated for pH, viscosity, thixotropic nature, IGF1 content and nasal toxicity study. The gel was stored under refrigerated condition.

The estimation of IGF-1 was done with Novum International ELISA kit. This assay employs an antibody specific for human IGF-BP-1 coated on a 96-well plate. Standards and samples were pipetted into the wells and IGF-BP-1 present in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human IGF-BP-1 antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added to the wells and color develops in proportion to the amount of IGF-BP-1 bound. The stop solution for the reaction changes the color from blue to yellow, and the intensity of the color was measured at 450 nm.

Viscosity of the gel was determined using Brook field viscometer (cone and plate model) and the shear stress at various rate of shear was also measured and the thixotropic nature of the gel was shown in Graph 4.1.

Nasal toxicity study was performed by treating nasal mucosa with gel, isopropyl alcohol and phosphate buffer saline pH6.4 separately followed by washing with PBS pH6.4. The microscopic images were taken and shown in Fig 4.17(O).

4.9 RESULTS

Table 4.1 Solubility of Clobazam in excipients

S.No	Excipients	By UV method (mg/ml)	order of UV curve	By HPLC method (mg/ml)
1.	Captex 100	7.65 ± 0.23	1st	6.24 ± 0.01
2.	Capmul MCM	15.97 ± 0.17	1st	16.01 ± 0.01
3.	Labrafac PG	7.85 ± 0.20	1st	6.64 ± 0.03
4.	Labrafac Lipophile WL1349	9.21 ± 0.05	1st	6.77 ± 0.01
5.	Peceol	1.01 ± 0.23	1st	1.62 ± 0.01
6.	Capryol 90	14.27 ± 0.09	1st	14.39 ± 0.14
7.	Labrafil M1944 Cs	7.94 ± 0.13	1st	8.74 ± 0.01
8.	Ethyl Oleate	5.49 ± 0.32	1st	3.02 ± 0.01
9.	Oleic acid	4.08 ± 0.35	1st	4.16 ± 0.17
10.	Transcutol P	43.66 ± 0.26	Zero order	44.99 ± 0.02
11.	Caprol 3GO	15.18 ± 0.91	1st	13.63 ± 0.01
12.	Caprol 10G-100	1.21 ± 0.78	1st	2.58 ± 0.003
13.	Ethanol	23.52 ± 0.17	Zero order	23.89 ± 0.03
14.	Capmul GMO–50EP/NF	7.50 ± 0.23	1st	7.78 ± 0.08
15.	Lauroglycol 90	7.21 ± 0.64	Zero order	7.93 ± 0.26
16.	Labrafac CC	6.98 ± 0.02	1st	7.14 ± 0.22
17.	Labrasol	18.35 ± 0.02	Zero order	18.70 ± 0.008
18.	Tween 80	12.37 ± 0.51	2nd	9.46 ± 0.11
19.	Tween 20	10.84 ± 0.19	2nd	7.76 ± 0.02
20.	PEG 200	25.78 ± 0.61	Zero order	26.23 ± 0.02
21.	PEG 400	12.11 ± 0.25	Zero order	12.73 ± 0.06
22.	Lauroglycol FCC	11.23 ± 0.41	1st	9.89 ± 0.04
23.	Captex 355 EP/NF	5.13 ± 0.92	1st	5.58 ± 0.01
24.	Caprol ET	4.59 ± 0.072	1st	3.97 ± 0.06
25.	Capmul MCM C10	7.6 ± 0.51	1st	7.43 ± 0.001
26.	Acconan CC6	15.87 ± 0.28	Zero order	15.95 ± 0.16
27.	Propylene Glycol	12.70 ± 0.03	1st	12.72 ± 0.02
28.	Isopropyl Palmitate	1.02 ± 0.53	1st	1.98 ± 0.003
29.	Isopropyl Myristate	1.85 ± 0.13	1st	2.43 ± 0.001
30.	Captex 8000	5.8 ± 0.32	1st	6.71 ± 0.002
31.	Capmul MCM C8	14.01 ± 0.15	1st	14.15 ± 0.27
32.	Captex 500	9.35 ± 0.02	1st	9.88 ± 0.02
33.	Labrafil M 2125	2.4 ± 0.15	1st	1.57 ± 0.01
34.	Capryol PGMC	11.81 ± 0.03	Zero order	11.12 ± 0.03

Table 4.2 Solubility of Clopidogrel bisulphate in excipients

S.No	Excipients	By UV method (mg/ml)	order of UV curve	By HPLC method (mg/ml)
1.	Captex 100	0.97 ± 0.02	1st	1.17 ± 0.02
2.	Capmul MCM	2.64 ± 0.11	1st	2.16 ± 0.014
3.	Labrafac PG	0.45 ± 0.31	1st	0.64 ± 0.01
4.	Labrafac Lipophile WL1349	0.083 ± 0.01	1st	0.077 ± 0.01
5.	Peceol	3.22 ± 0.24	1st	3.62 ± 0.002
6.	Capryol 90	2.23 ± 0.1	1st	2.64± 0.13
7.	Labrafil M1944 Cs	2.09 ± 0.21	1st	2.74 ± 0.0
8.	Ethyl Oleate	1.85 ± 0.05	1st	2.11 ± 0.04
9.	Oleic acid	9.54 ± 0.26	1st	9.6 ± 0.14
10.	Transcutol P	21.92 ± 0.34	Zero order	21.99 ± 0.02
11.	Caprol 3GO	3.12 ± 0.02	1st	3.63 ± 0.03
12.	Caprol 10G-100	0.81 ± 0.01	1st	1.058 ± 0.01
13.	Ethanol	53.86 ± 0.11	Zero order	53.89 ± 0.02
14.	Capmul GMO–50EP/NF	4.52 ± 0.07	1st	4.78 ± 0.03
15.	Lauroglycol 90	3.87 ± 0.01	Zero order	3.93 ± 0.02
16.	Labrafac CC	0.11± 0.01	1st	0.024 ± 0.38
17.	Labrasol	10.68 ± 0.15	Zero order	10.70 ± 0.01
18.	Tween 80	17.28 ± 0.41	2nd	19.46 ± 0.21
19.	Tween 20	14 .67 ± 0.62	2nd	14.96 ± 0.27
20.	PEG 200	18.12 ± 0.25	Zero order	19.43 ± 0.32
21.	PEG 400	7.92 ± 0.12	Zero order	8.34 ± 0.024
22.	Lauroglycol FCC	3.04 ± 0.32	1st	3.79 ± 0.06
23.	Captex 355 EP/NF	0.24 ± 0.12	1st	0.48 ± 0.18
24.	Caprol ET	0.14 ± 0.1	1st	0.37 ± 0.01
25.	Capmul MCM C10	0.28 ± 0.03	1st	0.43 ± 0.13
26.	Acconan CC6	4.21 ± 0.01	Zero order	4.75 ± 0.04
27.	Propylene Glycol	36.07 ± 0.24	1st	38.42 ± 0.017
28.	Isopropyl Palmitate	10.07 ± 0.21	1st	10.82 ± 0.04
29.	Isopropyl Myristate	13.85 ± 0.34	1st	14.24 ± 0.031
30.	Captex 8000	4.4 ± 0.13	1st	4.71 ± 0.015
31.	Capmul MCM C8	0.571 ± 0.25	1st	0.595 ± 0.13
32.	Captex 500	0.35 ± 0.16	1st	0.48 ± 0.041
33.	Labrafil M 2125	0.67 ± 0.015	1st	0.716 ± 0.01
34.	Capryol PGMC	1.04 ± 0.35	Zero order	1.262 ± 0.01

Fig 4.1 Phase diagram of CZ system 1(Capmul MCM, Acconan CC6 :Tween 20, Distilled Water)

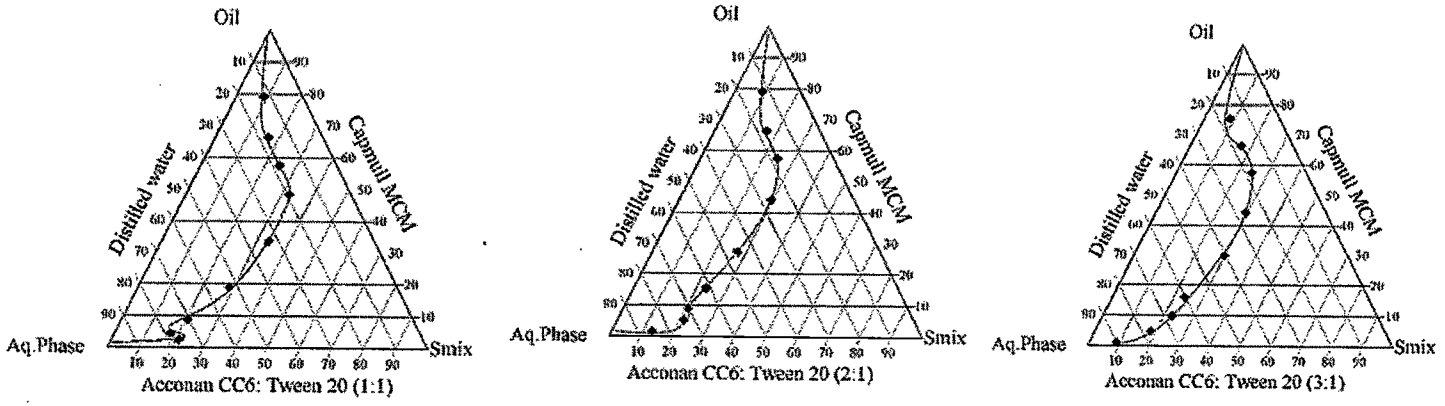


Fig 4.2 Phase diagram of CZ system 2(Capmul MCM, Tween 20: Transcutol P, Distilled Water)

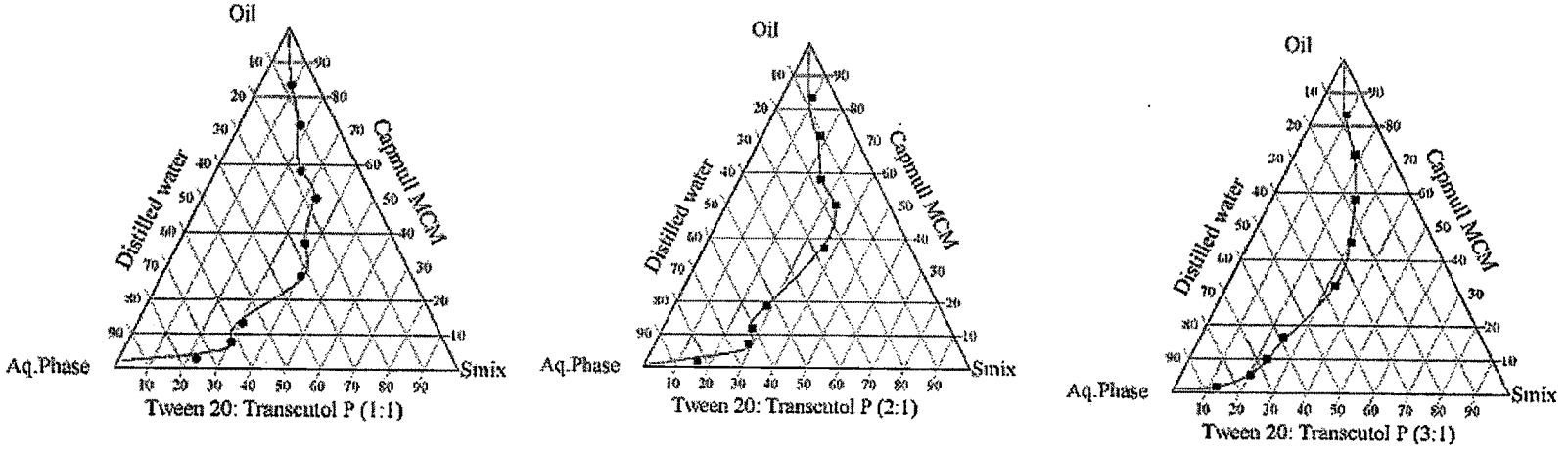


Table 4.3 CLOBAZAM System1: Capmul MCM, Acconan CC6:Tween 20 (3:1), Distilled Water

S. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)	%Transmittance(630nm)		Remarks
							As such	Dilution (1 in 10)	
1.	F1	2.5	20	77.5	--	--	--	--	Not soluble
2.	F2	2.5	30	67.5	-4.77	96.52	97.35	44.56	TOD
3.	F3	2.5	40	57.5	-4.22	92.0	101	52.50	TOD
4.	F4	2.5	50	47.5	-1.29	67.6	103.32	99.35	--
5.	F5	2.5	60	37.5	-5.03	24.36	99.38	99.7	--
6.	F6	5.0	20	75.0	-5.58	97.1	97.583	44.71	TOD
7.	**F7**	**5.0**	**30**	**65.0**	**-8.45**	**28.53**	**99.2**	**98.62**	--
8.	F8	5.0	40	55.0	-7.44	23.39	97.79	98.34	--
9.	F9	5.0	50	45.0	-4.4	19.68	96.72	97.99	--
10.	F10	5.0	60	35.0	-1.83	27.58	99.66	98.49	--
11.	F11	7.5	20	72.5	--	--	--	--	Not formed
12.	F12	7.5	30	62.5	-6.09	67.5	100.1	50.10	TOD
13.	F13	7.5	40	52.5	-5.76	38.73	100.9	98.91	--
14.	F14	7.5	50	42.5	-4.51	21.37	100.56	98.67	--
15.	F15	7.5	60	32.5	-1.31	17.24	100.5	99.35	--
16.	F16	10.0	20	70.0	--	--	--	--	Not formed
17.	F17	10.0	30	60.0	-3.34	99.7	98.79	37.01	TOD
18.	F18	10.0	40	50.0	--	--	--	--	Not formed
19.	F19	10.0	50	40.0	--	--	--	--	Not formed
20.	F20	10.0	60	30.0	--	--	--	--	Not formed

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

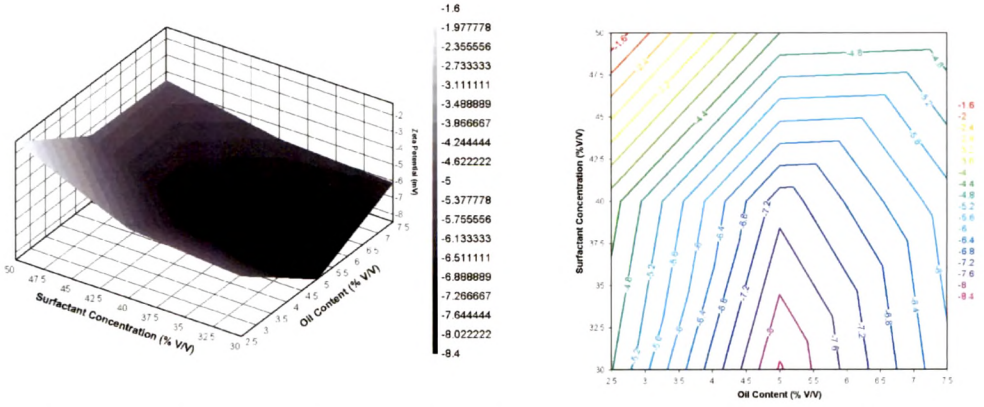
TOD - Turbid on dilution

Table 4.4 3^2 Factorial design for optimization of CZME system1

S.No	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)
1.	F2	2.5	30	67.5	-4.77	96.52
2.	F3	2.5	40	57.5	-4.22	92.0
3.	F4	2.5	50	47.5	-1.29	67.6
4.	**F7**	**5.0**	**30**	**65.0**	**-8.45**	**28.53**
5.	F8	5.0	40	55.0	-7.44	23.39
6.	F9	5.0	50	45.0	-4.4	19.68
7.	F12	7.5	30	62.5	-6.09	67.5
8.	F13	7.5	40	52.5	-5.76	38.73
9.	F14	7.5	50	42.5	-4.51	21.37

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

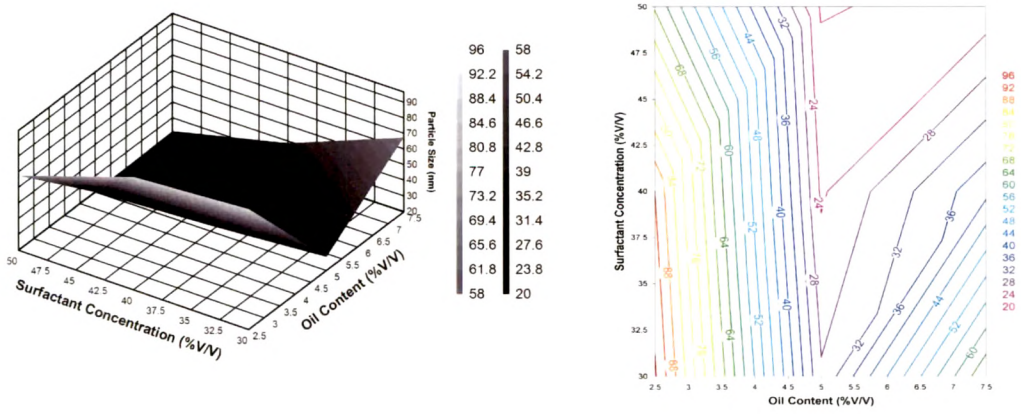
Fig 4.3 RSM and contour plots for zeta potential of CZ system1



Y1 = 8.3044 - 3.3627 **X1** – 0.4638 **X2** + 0.3717 **X11** + 0.0088 **X22** - 0.019 **X12**

(For zeta potential multiple R = 0.9858)

Fig 4.4 RSM and contour plots for Globule size of CZ system1



Y2 = 230.5756 – 65.8277 **X1** + 0.4008 **X2** + 6.4319 **X11** - 0.0117 **X22** - 0.1721 **X12**

(For globule size multiple R = 0.9760)

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 4.5 Effect of dilution on zeta potential and globule size measurement

S.No	Dilution	Zeta potential (mV)	Globule size(nm)
1.	Undiluted	--	39.21
2.	1 in 5	-11.88	32.31
3.	1 in 10	-7.12	23.58
4.	1 in 50	-17.8	65.3
5.	1 in 100	-32.4	138.5

Table 4.6 Checkpoint batches for CZ system1

S.No.	% v/v Oil	% v/v S_{mix}	Predicted ZP	Experimental ZP**	Predicted GS	Experimental GS**
1.	3.75	35	-7.03	-6.99* ± 2.33	51.28	60.74* ± 12.8
2.	3.75	45	-5.34	-5.12* ± 2.78	39.47	42.86* ± 8.7
3.	6.25	35	-7.80	-7.35* ± 3.4	32.45	28.51* ± 7.6
4.	6.25	45	-6.59	-5.98* ± 1.24	16.34	18.9* ± 6.3

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

** n = 3

Table 4.7 Muco adhesive Microemulsion of clobazam system1

S.No	Mucoadhesive agent	Concentration % W/V	Zeta potential* (mV)	Globule size* (nm)
1.	Carbopol P 940	0.1	-4.58 ± 3.4	18.74 ± 4.2
2.	"	0.2	-9.8 ± 1.8	19.52 ± 3.5
3.	"	0.5	-15.2 ± 3.46	19.79 ± 5.1
4.	Chitosan	0.1	1.44 ± 6.33	16.39 ± 4.23
5.	"	0.25	10.6 ± 5.56	19.92 ± 2.7
6.	"	0.5	14 ± 3.26	17.4 ± 2.1
7.	"	0.75	21.5 ± 14.1	21.92 ± 4.8

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

Table 4.8 CLOBAZAM System2: Capmul MCM, Tween 20: Transcutol P (3:1), Distilled Water

S. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)	%Transmittanceat630nm		Remarks
							As such	Dilution (1 in 10)	
1.	K1	2.5	20	77.5	-5.69	9.5	99.35	99.26	--
2.	K2	2.5	30	67.5	-8.79	9.604	99.57	99.13	--
3.	K3	2.5	40	57.5	-9.17	9.603	99.66	99.24	--
4.	**K4**	**2.5**	**50**	**47.5**	**-11.3**	**8.815**	**99.55**	**98.72**	--
5.	K5	2.5	60	37.5	-8.12	8.36	98.99	99.48	--
6.	K6	2.5	70	27.5	-8.94	8.5	99.69	98.91	--
7.	K7	5.0	20	75.0	-8.94	11.66	98.61	96.77	--
8.	K8	5.0	30	65.0	-7.31	11.29	99.44	98.4	--
9.	K9	5.0	40	55.0	-4.97	11.14	99.87	99.46	--
10.	K10	5.0	50	45.0	-7.73	9.455	99.73	95.97	--
11.	K11	5.0	60	35.0	-7.47	9.268	99.05	99.51	--
12.	K12	5.0	70	25.0	-7.24	9.081	98.11	99.66	--
13.	K13	7.5	20	72.5	-8.5	--	97.47	64.26	TOD
14.	K14	7.5	30	62.5	-10.8	12.38	99.32	96.86	--
15.	K15	7.5	40	52.5	-5.54	12.09	98.12	91.19	--
16.	K16	7.5	50	42.5	-11.3	11.05	100.2	90.53	--
17.	K17	7.5	60	32.5	-8.91	10.91	99.79	99.85	--
18.	K18	7.5	70	22.5	-5.91	10.36	100.7	99.71	--
19.	K19	10.0	20	70.0	--	--	--	--	Not formed
20.	K20	10.0	30	60.0	-7.66	127.6	101.8	68.35	TOD
21.	K21	10.0	40	50.0	-12.2	15.3	99.88	75.66	TOD
22.	K22	10.0	50	40.0	-4.39	12.8	98.35	99.17	--
23.	K23	10.0	60	30.0	-5.66	12.11	99.26	99.79	--
24.	K24	10.0	70	20.0	-11.4	12.13	99.18	98.65	--

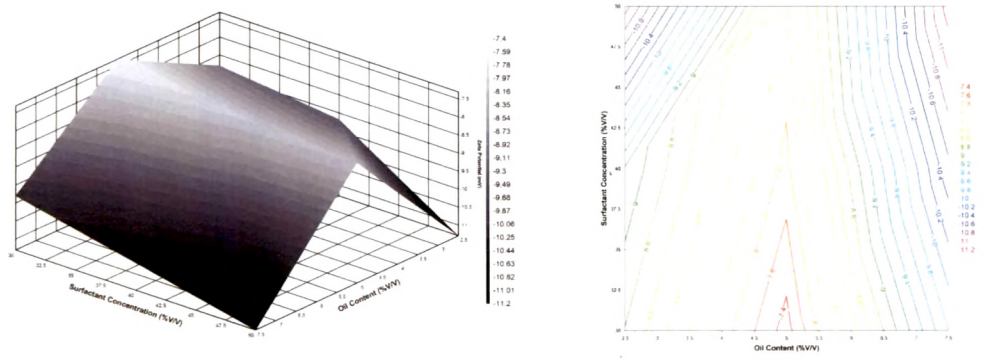
* Zeta potential and globule size were measured for the dispersion of 1 in 10 dilution in distilled water at 25°C
TOD - Turbid on dilution

Table 4.9 3^2 Factorial design for optimization of CZME system2

S.No	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)
1.	K2	2.5	30	67.5	-8.79	9.604
2.	K3	2.5	40	57.5	-9.17	9.603
3.	**K4**	**2.5**	**50**	**47.5**	**-11.3**	**8.815**
4.	K8	5	30	65.0	-7.31	11.29
5.	K9	5	40	55.0	-7.73	11.14
6.	K10	5	50	45.0	-7.97	9.455
7.	K14	7.5	30	62.5	-9.8	12.38
8.	K15	7.5	40	52.5	-10.54	12.09
9.	K16	7.5	50	42.5	-11.3	11.05

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

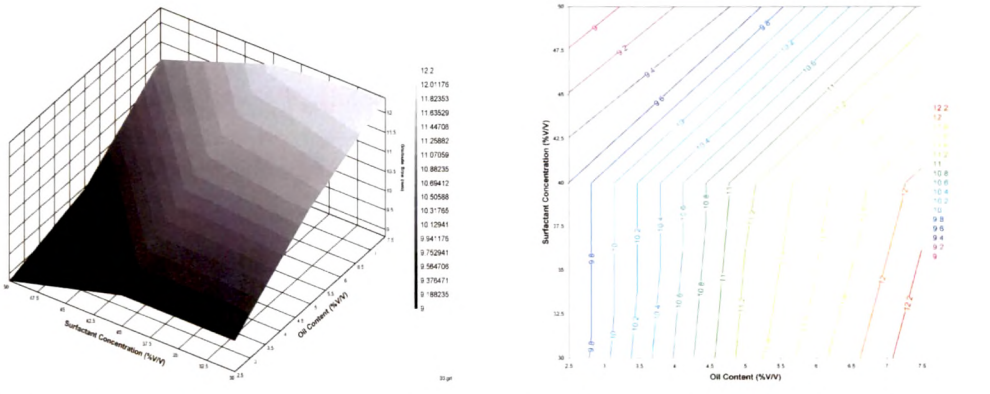
Fig 4.5 RSM and contour plots for zeta potential of CZ system2



Y1 = -15.7267 + 3.4053 **X1** + 0.0837 **X2** - 0.3968 **X11** - 0.0026 **X22** +0.0101 **X12**

(For zeta potential multiple R = 0.9728)

Fig 4.6 RSM and contour plots for Globule size of CZ system2



Y2 = 1.6803 + 0.7771 **X1** + 0.3708 **X2** – 0.0061 **X11** - 0.0051 **X22** - 0.0054 **X12**

(For globule size multiple R = 0.9896)

Table 4.10 Checkpoint batches for CZ system2

S.No.	% v/v Oil	% v/v S_{mix}	Predicted ZP	Experimental ZP**	Predicted GS	Experimental GS**
1.	3.75	35	-7.53	-7.25* ± 2.41	10.53	10.51* ± 4.2
2.	3.75	45	-8.39	-7.99 * ± 5.35	9.96	10.23 * ± 3.5
3.	6.25	35	-7.99	-7.35 * ± 4.31	13.47	12.89 * ± 2.8
4.	6.25	45	-8.60	-8.5* ± 2.16	11.14	12.11 * ± 4.7

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

** n = 3

Table 4.11 Muco adhesive Microemulsion of clobazam system2

S.No	Mucoadhesive agent	Concentration % W/V	Zeta potential* (mV)	Globule size* (nm)
1.	Carbopol P 940	0.1	-7.24 ± 3.5	13.56 ± 3.2
2.	"	0.2	-12.58 ± 7.3	12.01 ± 4.1
3.	"	0.3	-17.1 ± 4.5	11.35 ± 7.1
4.	Chitosan	0.1	3.5 ± 4.2	10.75 ± 6.3
5.	"	0.25	11.89 ± 3.7	11.21 ± 3.2
6.	"	0.5	19.54 ± 5.6	14.8 ± 2.1

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

Table 4.12 Optimized clobazam formulations

S.No	Test	CZME1	CZME11	CZMMME12	CZME2	CZMME21	CZMME22
1	Zeta potential(mV)	-8.45±5.1	-15.2±3.5	21.5 ± 14.1	-11.3±4.2	-17.1 ± 4.5	19.54 ± 5.6
2	Globule size(nm)	16.47±5.4	19.79±5.1	21.92 ± 4.8	8.815±3.5	11.35 ± 7.1	14.8 ± 2.1
3	%Transmittance	99.2±0.4	--	--	99.55±0.3	--	--
4	pH at 30°C	5.68±0.23	5.43± 0.2	5.58±0.12	5.31±0.11	4.65±0.14	5.31±0.2
5	Assay (%)	99.35±0.5	99.19±0.3	98.34±0.4	99.51±0.4	100.2±0.25	99.1±0.3
6	Viscosity at 33°C (cP)	7.73±0.43	25.8±0.71	23.2±1.1	5.35±0.87	19.17±0.54	16.23±0.6

Table 4.13 Stability and solubility of clopidogrel bisulphate in buffers

S. No	pH	Acetate buffer (µg/ml)				Phosphate buffer(µg/ml)			
		0 hr	0.5hr	1 hr	2hr	0 hr	0.5hr	1 hr	2hr
1.	2	198.71	171.61	155.88	156.56	165.26	153.90	135.14	128.24
2.	3	149.89	151.17	143.14	129.54	140.42	155.69	153.40	143.97
3.	4	89.06	53.59	40.12	36.33	78.32	69.28	63.35	54.39
4	5	38.88	37.15	36.97	35.25	53.28	48.76	47.21	46.44
5.	6	26.53	25.78	25.63	22.73	51.41	49.53	48.78	48.51

Fig 4.7 Phase diagram of CS system 2(Capmul GMO, Tween 20: Transcutol P, Acetate buffer pH5)

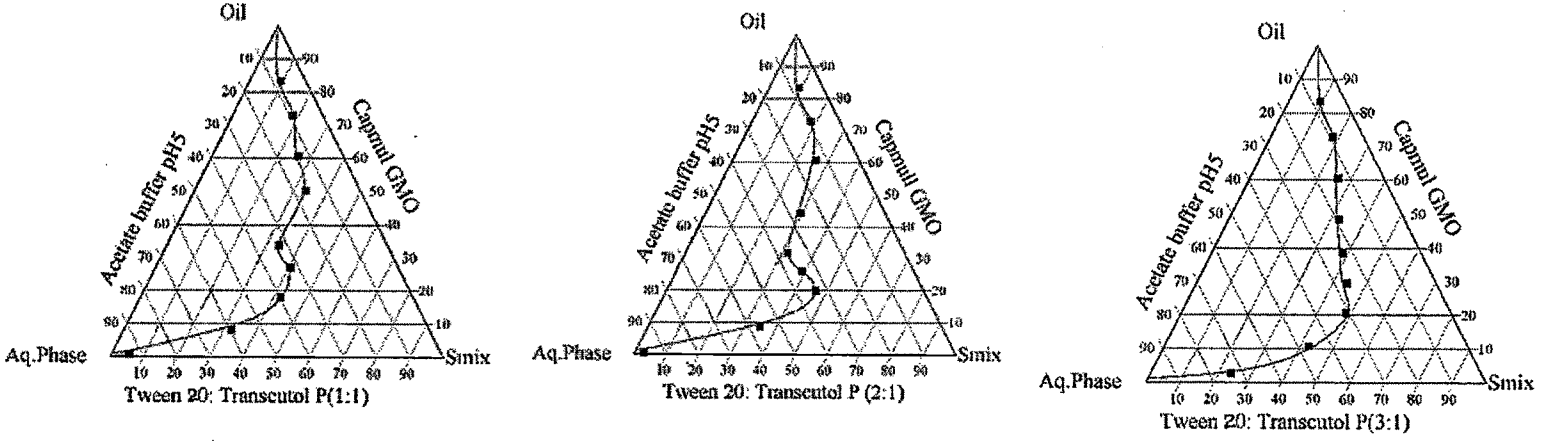


Fig 4.8 Phase diagram of CS system 3(Capmul GMO, Tween 20: PEG200, Acetate buffer pH5)

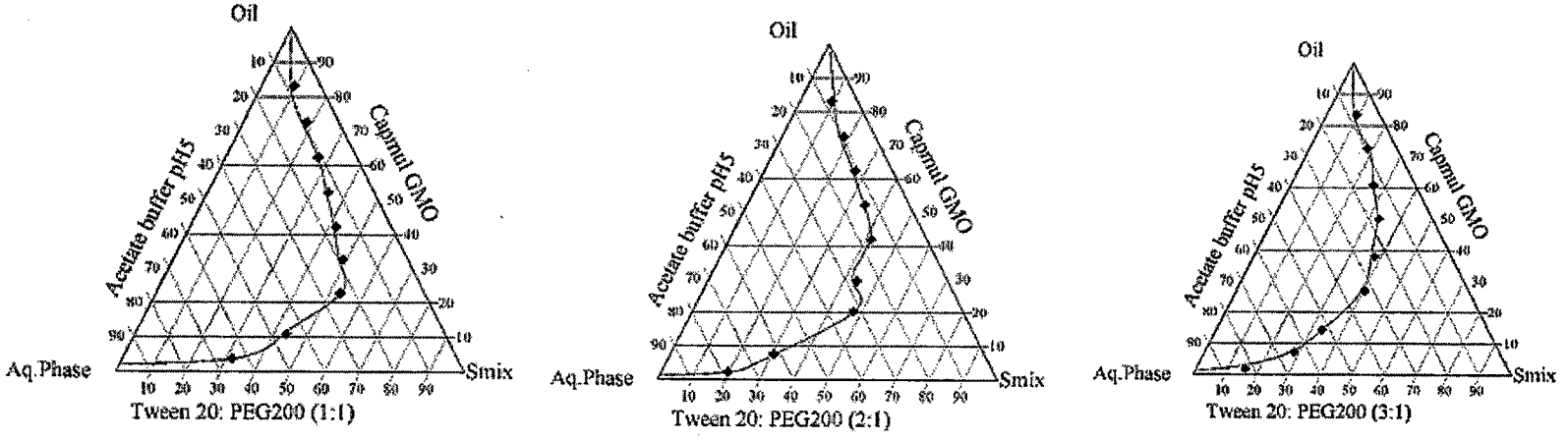


Table 4.14 Clopidogrel Bisulphate system1: Capmul GMO, Tween 80: Transcutol P(2:1), Distilled water

S. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)	Transmittance at 630nm		pH at 30 °C	Remarks
							Undiluted	Dilution (1 in 10)		
1.	R1	2.5	30	67.5	-2.29	21.6	99.34	99.99	2.407	--
2.	R2	2.5	40	57.5	-6.16	16.3	99.56	99.01	2.524	--
3.	**R3**	**2.5**	**50**	**47.5**	**-6.28**	**13.51**	**100.69**	**99.17**	**2.579**	--
4.	R4	2.5	60	37.5	-5.58	11.17	99.93	100.2	2.73	--
5.	R5	5.0	30	65.0	-1.11	30.5	--	--	--	Not formed
6.	R6	5.0	40	55.0	-2.99	19	98.30	99.2	2.481	--
7.	R7	5.0	50	45.0	-3.3	17.25	99.33	99.68	2.62	--
8.	R8	5.0	60	35.0	-5.78	11.36	101	100.2	2.65	--
9.	R9	7.5	30	62.5	--	--	--	--	--	Not formed
10.	R10	7.5	40	52.5	0.393	29.6	98.36	98.45	2.501	--
11.	R11	7.5	50	42.5	-3.84	18.04	99.21	100.2	2.75	--
12.	R12	7.5	60	32.5	-5.64	14.33	99.63	99.45	3.335	--
13.	R13	10.0	30	60.0	--	--	--	--	--	Not formed
14.	R14	10.0	40	50.0	--	--	--	--	--	Not formed
15.	R15	10.0	50	40.0	0.148	235.1	98.24	65.21	2.823	TOD
16.	R16	10.0	60	30.0	0.266	132.7	97.17	72.41	3.253	TOD

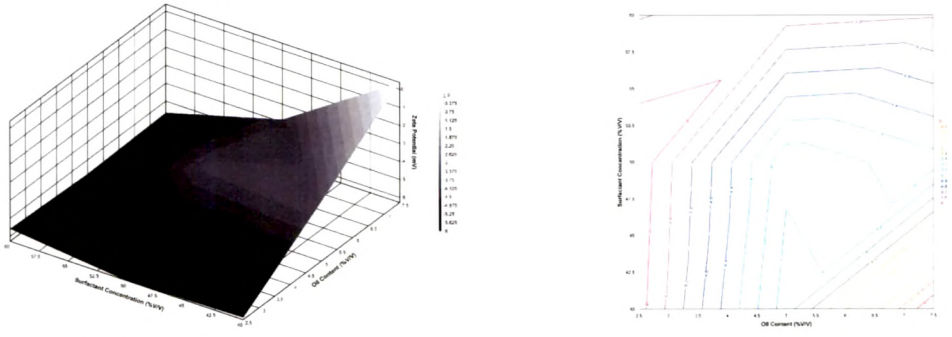
* Zeta potential and globule size were measured for the dispersion of 1 in 10 dilution in distilled water at 25°C
TOD - Turbid on dilution

Table 4.15 3^2 Factorial design for optimization of CSME system1

S.No	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)
1.	R2	2.5	40	57.5	-6.16	16.3
2.	**R3**	**2.5**	**50**	**47.5**	**-6.28**	**13.51**
3.	R4	2.5	60	37.5	-5.58	11.17
4.	R6	5	40	55	-2.99	19
5.	R7	5	50	45	-3.3	17.25
6.	R8	5	60	35	-5.78	11.36
7.	R10	7.5	40	52.5	0.393	29.6
8.	R11	7.5	50	42.5	-3.84	18.04
9.	R12	7.5	60	32.5	-5.64	14.33

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

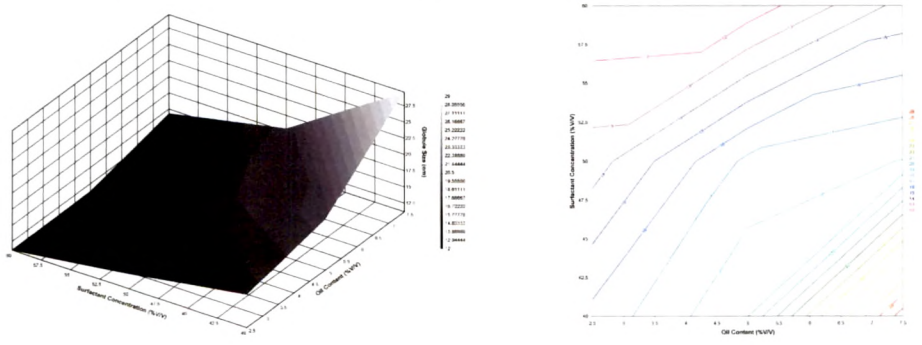
Fig 4.9 RSM and contour plots for zeta potential of CS system1



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

(For zeta potential multiple R = 0.9757)

Fig 4.10 RSM and contour plots for Globule size of CS system1



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

(For globule size multiple R = 0.9707)

Table 4.16 Clopidogrel Bisulphate system2: Capmul GMO, Tween 20: Transcutol P (2:1), Acetate Buffer (pH 5)

S. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)	Transmittance at 630nm		pH at 30 °C	Remarks
							Undiluted	Dilution (1 in 10)		
1.	H1	2.5	30	67.5	-0.988	16.14	99.82	89.99	5.03	--
2.	H2	2.5	40	57.5	-1.03	18.47	98.66	99.19	5.24	--
3.	H3	2.5	50	47.5	-3.15	20.54	99.79	101.1	5.38	--
4.	H4	2.5	60	37.5	-3.62	19.35	99.93	100.2	5.60	--
5.	**H5**	**2.5**	**70**	**27.5**	**-3.98**	**17.17**	**100.1**	**99.98**	**5.85**	--
6.	H6	5.0	30	65.0	--	--	--	--	--	Not formed
7.	H7	5.0	40	55.0	-2.16	22.78	99.30	99.2	5.22	--
8.	H8	5.0	50	45.0	-2.4	19.93	98.33	100.1	5.42	--
9.	H9	5.0	60	35.0	-2.65	18.16	100.1	100.2	5.59	--
10.	H10	5.0	70	25.0	-3.15	17.33	100.4	101.9	5.67	--
11.	H11	7.5	30	62.5	--	--	--	--	--	Not formed
12.	H12	7.5	40	52.5	--	--	99.23	35.08	5.25	TOD
13.	H13	7.5	50	42.5	-2.28	27.95	99.76	79.02	5.41	--
14.	H14	7.5	60	32.5	-2.34	22.98	99.68	99.8	5.60	--
15.	H15	7.5	70	22.5	-2.94	20.35	99.96	101.3	5.82	--
16.	H16	10.0	30	60.0	--	--	--	--	--	Not formed
17.	H17	10.0	40	50.0	--	--	--	--	--	Not formed
18.	H18	10.0	50	40.0	--	--	99.93	15.36	5.44	TOD
19.	H19	10.0	60	30.0	-1.24	18.6	98.90	8.52	5.87	TOD
20.	H20	10.0	70	20.0	-0.988	16.14	100.2	89.70	6.03	Slightly turbid

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

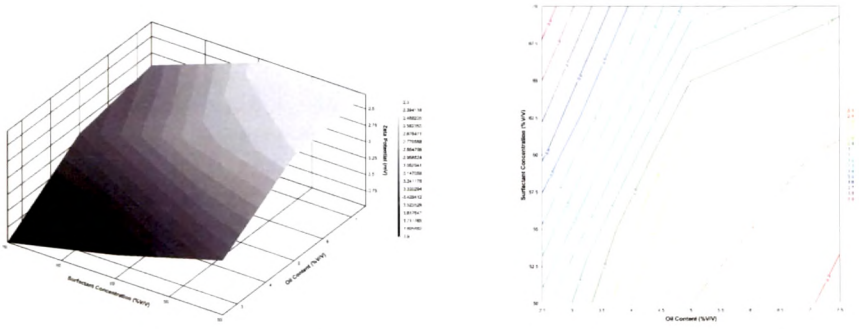
TOD - Turbid on dilution

Table 4.17 3^2 Factorial design for optimization of CSME system2

S.No	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)
1.	H3	2.5	50	47.5	-3.15	20.54
2.	H4	2.5	60	37.5	-3.62	19.35
3.	**H5**	**2.5**	**70**	**27.5**	**-3.98**	**17.17**
4.	H8	5.0	50	45.0	-2.4	19.93
5.	H9	5.0	60	35.0	-2.65	18.16
6.	H10	5.0	70	25.0	-3.15	17.33
7.	H13	7.5	50	42.5	-2.28	27.95
8.	H14	7.5	60	32.5	-2.34	22.98
9.	H15	7.5	70	22.5	-2.94	20.35

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

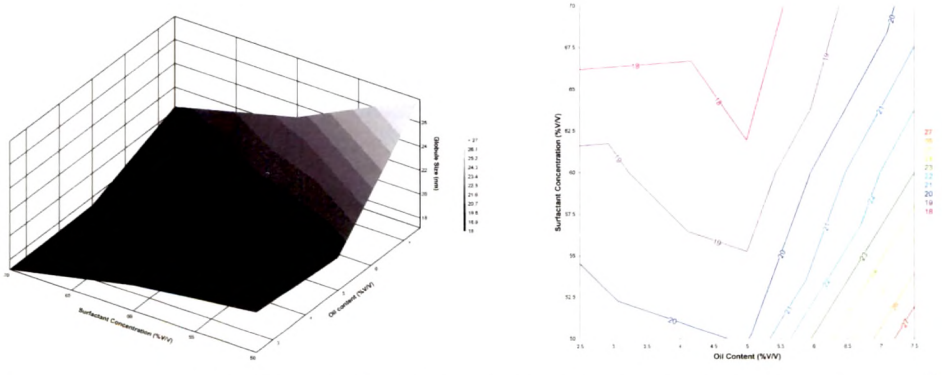
Fig 4.11 RSM and contour plots for zeta potential of CS system2



$$\mathbf{Y1} = -6.7012 + 0.722\,\mathbf{X1} + 0.09867\,\mathbf{X2} - 0.0555\,\mathbf{X11} - 0.0012\,\mathbf{X22} + 1.25\times 10^{-6}\,\mathbf{X12}$$

(For zeta potential multiple R = 0.9934)

Fig 4.12 RSM and contour plots for Globule size of CS system2



$$\mathbf{Y2} = 48.4319 - 3.7187\,\mathbf{X1} - 0.6842\,\mathbf{X2} + 0.6046\,\mathbf{X11} + 0.0049\,\mathbf{X22} - 3.8\times 10^{-5}\,\mathbf{X12}$$

(For globule size multiple R = 0.9834)

Table 4.18 Checkpoint batches for CS system2

S.No.	% v/v Oil	% v/v S_{mix}	Predicted ZP	Experimental ZP**	Predicted GS	Experimental GS*
1.	3.75	55	-2.98	-2.51* ± 1.2	20.17	22.7 * ± 4.1
2.	3.75	65	-3.43	-3.78 * ± 2.3	19.21	20.5 * ± 3.4
3.	6.25	55	-2.56	-2.14 * ± 1.5	25.99	27.3 * ± 5.2
4.	6.25	65	-3.01	-3.42 * ± 2.6	25.02	27.5 * ± 3.8

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

** n = 3

Table 4.19 Muco adhesive Microemulsion of clopidogrel bisulphate system 2:

S.No	Mucoadhesive agent	Concentration % W/V	Zeta potential* (mV)	Globule size* (nm)	pH at 30°C
1.	Carbopol P 940	0.0625	-5.38 ± 3.21	20.72 ± 5.2	5.59
2.	"	0.125	-10.7 ± 4.1	18.89 ± 2.1	5.49
3.	"	0.1875	-11.2 ± 4.3	17.93 ± 4.5	5.34
4.	"	0.25	-13.0 ± 3.54	19.77 ± 5.1	5.42
5.	Chitosan	0.125	8.67 ± 4.94	11.78 ± 1.7	5.66
6.	"	0.25	13.0 ± 2.85	10.37 ± 6.2	5.16
7.	"	0.5	18.1 ± 2.94	11.23 ± 4.4	5.35
8.	"	0.75	21.7 ± 4.78	11.20 ± 5.1	4.05

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

Table 4.20 Clopidogrel Bisulphate system3: Capmul GMO,Tween 20:PEG 200(3:1), Acetate Buffer (pH 5)

S. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)	%Transmittance at 630nm		pH at 30 °C	Remarks
							Undiluted	Dilution (1 in 10)		
1.	A1	2.5	20	77.5	-3.63	14.4	99.65	99.58	5.210	
2.	A2	2.5	30	67.5	-1.79	11.43	100.1	99.42	5.259	--
3.	**A3**	**2.5**	**40**	**57.5**	**-7.18**	**13.73**	**99.91**	**99.52**	**5.513**	**--**
4.	A4	2.5	50	47.5	-4.49	12.14	99.39	99.61	5.63	--
5.	A5	2.5	60	37.5	-3.22	10.08	98.67	99.54	5.793	
6.	A6	3.75	20	76.25	--	--	--	--	--	Not formed
7.	A7	3.75	30	66.25	--	--	--	--	--	Not formed
8.	A8	3.75	40	56.25	-4.88	12.11	99.41	99.92	5.426	--
9.	A9	3.75	50	46.25	-3.77	10.459	99.98	99.42	5.56	--
10.	A10	3.75	60	36.25	-3.22	8.89	99.58	99.83	5.74	--
11.	A11	5	30	65	--	--	--	--	--	Not formed
12.	A12	5	40	55	-3.92	11.78	98.87	100.2	5.337	--
13.	A13	5	50	45	-3.77	10.64	99.29	99.43	5.538	--
14.	A14	5	60	35	-3.52	10.41	99.57	100.1	5.726	--
15.	A15	7.5	30	62.5	--	--	--	--	--	Not formed
16.	A16	7.5	40	52.5	-2.15	195.8	98.21	55.46	5.4	TOD
17.	A17	7.5	50	42.5	-2.18	17.19	99.36	82.41	5.93	TOD

* Zeta potential and globule size were measured for the dispersion of 1 in 5 dilution in distilled water at 25°C

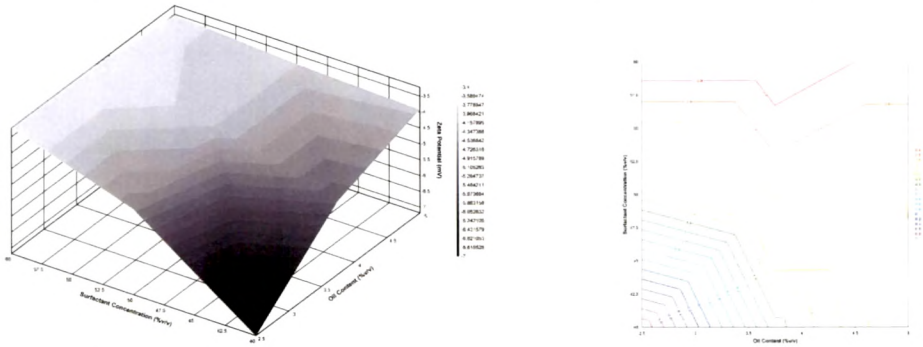
TOD - Turbid on dilution

Table 4.21 3^2 Factorial design for optimization of CSME system3

S.No	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	Zeta potential* (mV)	Globule size* (nm)
1.	**A3**	**2.5**	**40**	**47.5**	**-7.18**	**13.73**
2.	A4	2.5	50	37.5	-4.49	12.14
3.	A5	2.5	60	27.5	-3.22	10.08
4.	A8	3.75	40	56.25	-4.88	12.11
5.	A9	3.75	50	46.25	-3.77	10.45
6.	A10	3.75	60	36.25	-3.22	8.89
7.	A12	5.0	40	45.0	-3.92	11.78
8.	A13	5.0	50	35.0	-3.77	10.64
9.	A14	5.0	60	25.0	-3.52	10.41

*Zeta potential and globule size were measured for the dispersion of 1 in 5 dilution in distilled water at 25°C

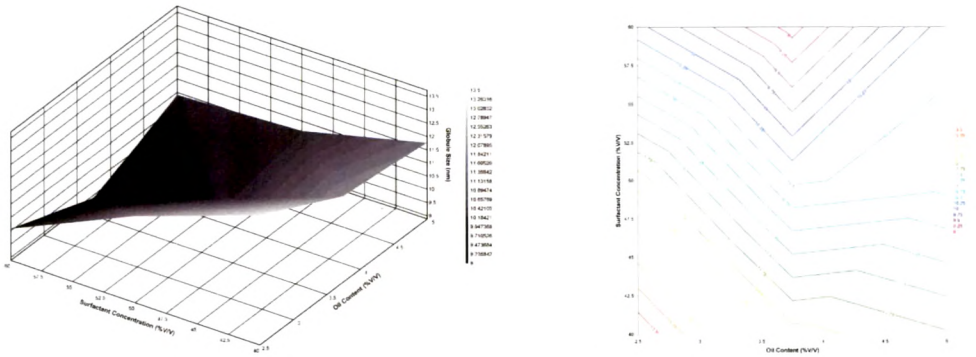
Fig 4.13 RSM and contour plots for zeta potential of CS system3



Y1 = -35.3278 + 5.9387 **X1** + 0.6807 **X2** -0.2517 **X11** - 0.0031 **X22** – 0.0712 **X12**

(For zeta potential multiple R = 0.9884)

Fig 4.14 RSM and contour plots for Globule size of CS system3



Y2 = 38.373 – 7.3856 **X1** - 0.3953 **X2** + 0.6253 **X11** + 0.0009 **X22** + 0.0456 **X12**

(For globule size multiple R = 0.9899)

Table 4.22 Checkpoint batches for CS system3

S.No.	% v/v Oil	% v/v S_{mix}	Predicted ZP	Experimental ZP**	Predicted GS	Experimental GS*
1.	3.125	45	-4.89	-5.01* ± 2.0	11.79	13.2 * ± 4.1
2.	3.125	55	-3.41	-3.99 * ± 4.1	10.13	11.01* ± 2.4
3.	4.375	45	-3.83	-3.65 * ± 1.4	10.98	11.17 * ± 5.1
4.	4.375	55	-3.24	-2.99 * ± 2.3	9.89	8.7 * ± 4.3

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

** n = 3

Table 4.23 Muco adhesive Microemulsion of clopidogrel bisulphate system3

S.No	Mucoadhesive agent	Concentration % W/V	Zeta potential* (mV)	Globule size* (nm)	pH at 30°C
1.	Carbopol P 940	0.1	-7.2 ± 4.1	11.92 ± 3.8	5.32
2.	"	0.2	-12.4 ± 3.7	13.29 ± 2.4	5.17
3.	"	0.5	-22.5 ± 4.6	13.49 ± 4.3	5.02
4.	Chitosan	0.25	19.7 ± 3.52	13.21 ± 5.2	5.52
5.	"	0.5	21.8 ± 3.42	15.78 ± 2.3	5.36
6.	"	1	27.0 ± 3.21	13.69 ± 1.8	5.27

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

Table 4.24 Optimized clopidogrel bisulphate formulations

S. No	Test	CSME2	CSME21	CSMME22	CSME3	CSMME31	CSMME32
1	Zeta potential(mV)	-3.98±3.4	-13.0±3.5	21.7 ± 4.78	-7.18±5.1	-22.5 ± 4.6	27.0 ± 3.21
2	Globule size(nm)	17.17±4.7	19.77±5.1	11.20 ± 5.1	13.73±2.6	13.49 ± 4.3	13.69 ± 1.8
3	%Transmittance	100.1±0.2	--	--	99.91±0.3	--	--
4	pH at 30°C	5.85±0.23	5.42± 0.1	4.05±0.12	5.513±0.11	5.02±0.17	5.27±0.24
5	Assay (%)	99.4±0.3	99.25±0.5	98.71±0.3	99.12±0.1	99.31±0.5	99.42±0.3
6	Viscosity at 33°C (cP)	5.53±0.11	18.3±0.2	21.3±0.3	7.37±0.41	26.7±0.21	21.43±0.4

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

Fig.4.15 TEM of CZME1

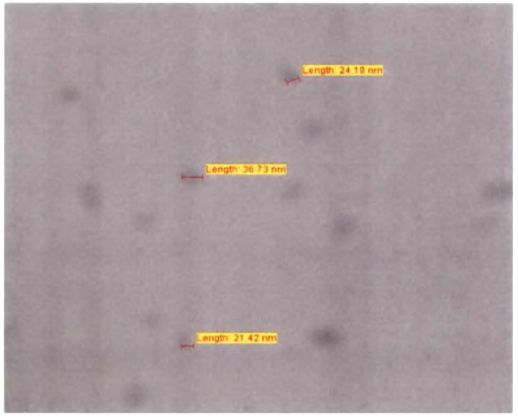


Fig.4.16 TEM of CSME3

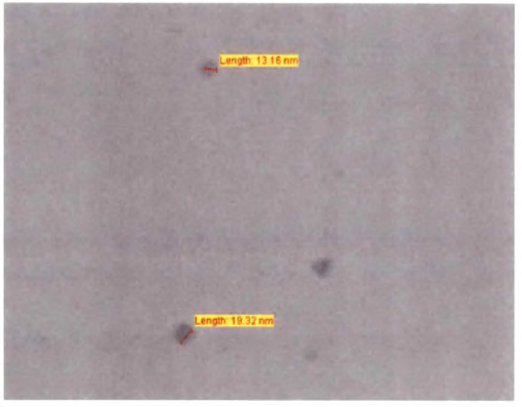


Fig 4.17 Optical microscopy images of sheep nasal mucosa treated with formulations

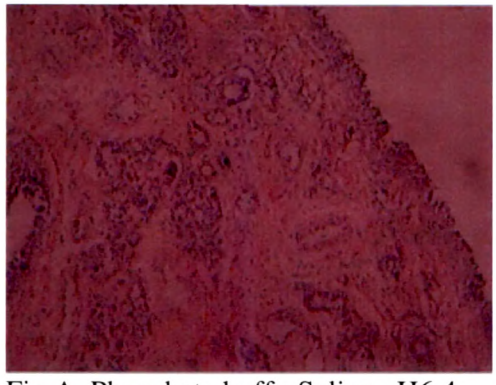


Fig.A. Phosphate bufferSaline pH6.4

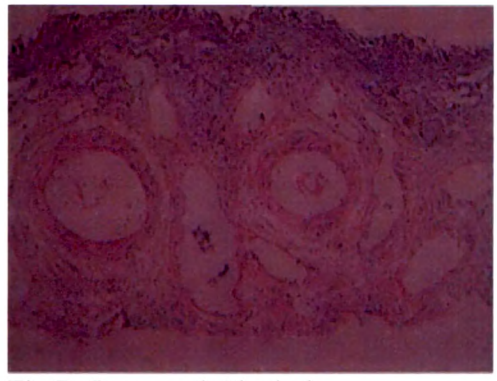


Fig.B. Isopropyl Alcohol

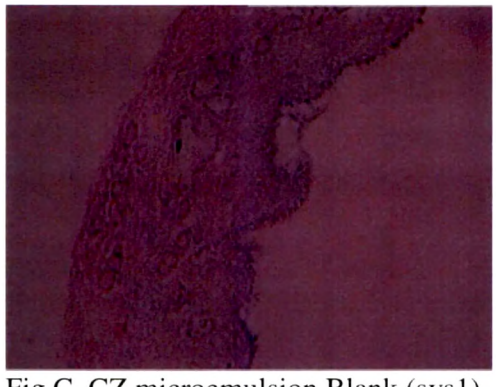


Fig.C. CZ microemulsion Blank (sys1)

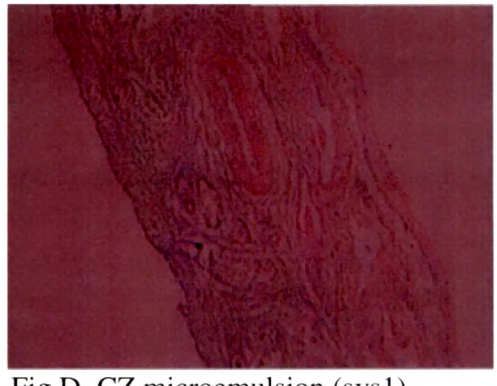


Fig.D. CZ microemulsion (sys1)

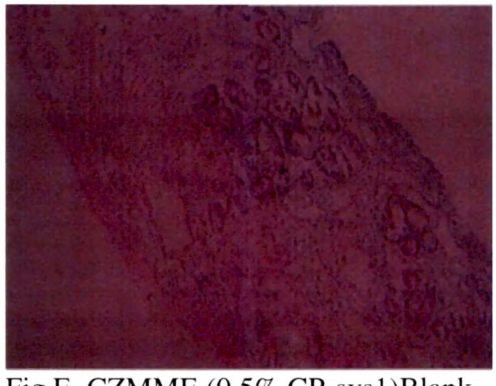


Fig.E. CZMME (0.5% CP-sys1)Blank

Fig.F. CZMME (0.5% CP-sys1)

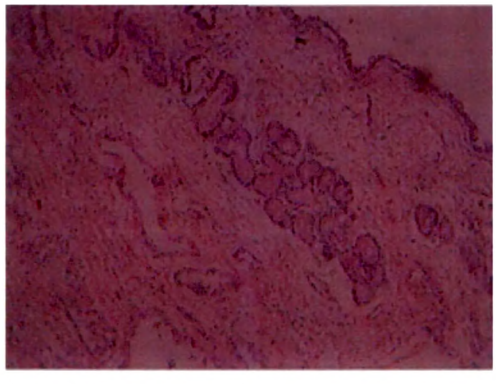


Fig.G. CZ solution Blank

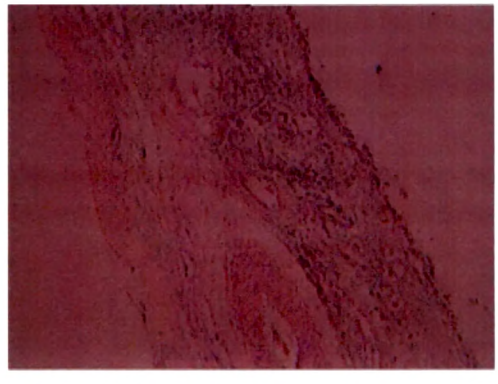


Fig.H. CZ solution

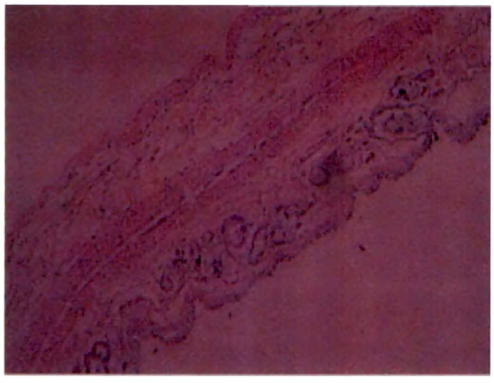


Fig.I. CS microemulsion Blank (sys3)

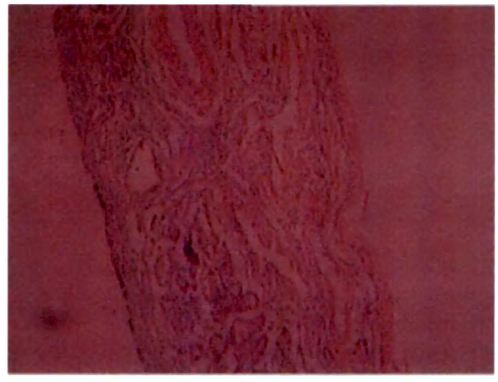


Fig.J. CS microemulsion (sys3)

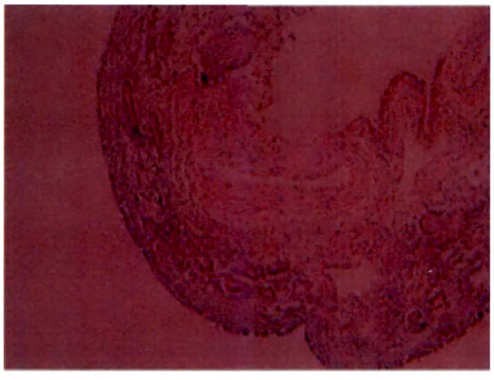


Fig.K. CSMME (0.5% CP-sys3)Blank

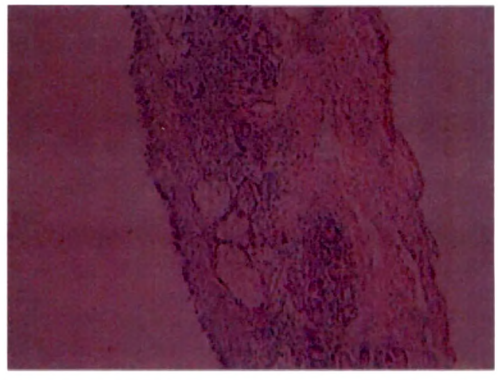


Fig.L. CSMME (0.5% CP-sys3)

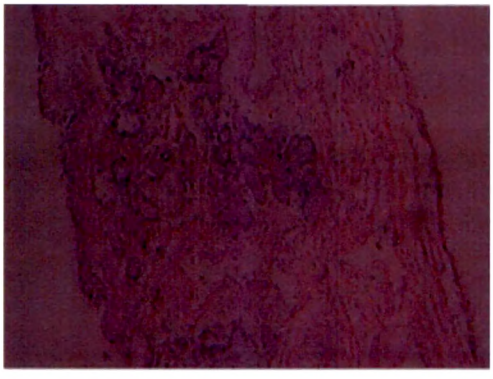


Fig.M. CS solution Blank

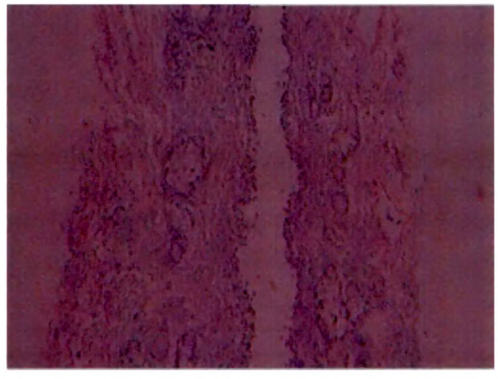


Fig.N. CS solution

Fig.O. Gel

Table 4.25 Stability study of Clobazam microemulsions

S. No	System	Period (month)	Room temperature				Cold storage			
			Zeta potential (mV)*	Globule size(nm)*	% trans mittance	%drug content	Zeta potential (mV)*	Globule size(nm)*	% trans mittance	%drug content
1.	CZME1	0	-8.45 ±5.1	16.47±5.4	99.2±0.4	99.35±0.5	-8.45 ±5.1	16.47±5.4	99.2±0.4	99.35±0.5
		1	-8.67 ± 3.4	16.34±2.8	99.52±0.5	99.41±0.4	-8.2 ±2.3	17.72±3.2	100.3±0.5	97.41±0.4
		2	-7.98 ± 4.6	17.75±3.4	99.84±0.1	99.47±0.2	-8.51 ± 3.6	17.5±2.2	99.72±0.2	99.37±02
		4	-8.12 ± 3.7	17.54±2.4	100.5±0.4	99.6±0.2	-8.88 ± 2.4	18.64±4.2	99.81±0.1	98.41±0.3
		6	-9.12 ± 6.2	19.14±3.2	99.82±0.3	98.17±0.7	-9.5 ± 3.2	18.91±2.5	99.42±0.2	98.35±0.4
2.	CZME2	0	-11.3 ± 4.4	8.81±3.5	99.55±0.4	99.51±0.4	-11.3 ± 4.4	8.81±3.5	99.55±0.4	98.51±0.4
		1	-13.4 ± 5.3	8.74±3.5	99.3±0.1	99.63±0.6	-12.4 ± 3.1	9.65±5.3	99.52±0.3	98.42±0.4
		2	-10.91± 3.5	9.65±4.2	99.74±0.4	99.41±0.2	-11.3± 4.2	10.35±7.1	98.91±0.5	98.54±0.3
		4	-12.7 ± 6.1	11.44±5.2	99.23±0.6	99.24±0.2	-13.2 ± 1.7	10.81±2.6	99.41±0.4	98.21±0.4
		6	-13.8 ± 2.1	12.51±4.2	97.64±0.3	99.35±0.4	-13.1 ± 3.7	11.73±4.5	99.27±0.1	98.17±0.4

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

Table 4.26 Stability study of Clopidogrel bisulphate microemulsions

System	Period (month)	Room temperature					Cold storage				
		Zeta potential (mV)*	Globule size(nm)*	% trans mittance	% drug content	pH	Zeta potential (mV)*	Globule size(nm)*	% trans mittance	% drug content	pH
CSME2	0	-3.98±2.1	17.17±5.2	100.1±0.2	99.4±0.3	5.85±0.2	-3.98±2.1	17.17±5.2	100.1±0.2	99.4±0.3	5.85±0.2
	1	-4.13±5.2	15.34±7.5	99.35±0.5	98.24±0.5	5.75±0.2	-4.21±5.3	15.72±4.1	99.73±0.5	99.24±0.2	6.01±0.2
	2	-4.52±3.4	18.27±3.4	98.84±0.3	99.45±0.7	5.66±0.3	-3.57±4.3	19.65±3.2	97.62±0.4	99.31±0.7	5.72±0.2
	4	-5.67±7.3	22.54±4.6	99.51±0.4	98.56±1.1	5.17±0.2	-4.71±3.6	18.64±8.1	98.1±0.8	98.42±0.6	5.34±0.1
	6	-6.17±4.5	25.41±8.1	97.22±0.5	96.11±0.8	4.06±0.2	-4.53±4.9	20.61±2.5	99.24±0.1	97.53±0.3	5.29±0.3
CSME3	0	-7.18±2.7	13.73±4.5	99.91±0.4	99.12±0.5	5.51±0.1	-7.18±2.7	13.73±4.5	99.91±0.4	99.12±0.5	5.51±0.1
	1	-8.21±3.8	14.24±5.1	101.2±0.1	98.65±0.6	5.75±0.4	-8.01±5.1	14.65±5.2	99.25±0.5	98.62±0.4	5.61±0.3
	2	-7.94 ±1.5	13.65±3.2	99.67±0.8	99.01±0.4	5.25±0.3	-8.17±4.2	13.33±7.1	98.39±0.3	98.24±0.3	5.34±0.1
	4	-8.54±6.3	17.24±4.1	98.82±0.6	98.71±0.2	5.34±0.3	-7.65±2.1	15.68±2.8	99.01±0.4	98.01±0.3	5.41±0.3
	6	-9.24±3.2	19.25±6.2	97.86±0.5	97.53±0.1	5.17±0.2	-8.39±5.6	15.63±5.0	99.17±0.2	98.17±0.4	5.25±0.2

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

Table 4.27 Accelerated Stability Study

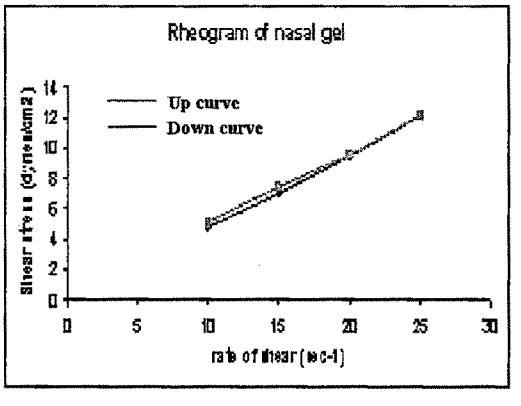
S. No	Parameters	Empty CZ ME	CZ ME1	Empty CS ME	CS ME3
		Before ASS			
1.	ZP	-9.69 ± 3.34	-8.45 ± 5.05	-4.19 ± 6.4	-7.18 ± 3.1
2.	GS	12.25 ± 4.9	16.47 ± 5.4	12.59 ± 3.5	13.73 ± 4.8
3	%T	99.6 ± 0.3	99.2 ± 0.4	98.9 ± 1.2	99.91± 0.65
		After Centrifugation			
4.	ZP	-10.27 ± 2.4	-8.94 ± 3.12	-5.24 ± 1.2	-6.8 ± 4.3
5.	GS	14.23 ± 3.4	19.24 ± 3.2	17.68 ± 4.8	15.25 ± 5.6
6.	%T	99.98 ± 0.5	99.56 ± 0.91	97.5 ± 0.95	99.14 ± 0.8
		After Freeze thaw cycle			
7.	ZP	-10.17± 5.21	-9.17 ± 2.62	-5.81 ± 2.6	-8.27 ± 3.1
8.	GS	11.35 ± 5.4	17.65 ± 5.32	11.26 ± 5.3	11.65 ± 6.7
9.	%T	98.76 ± 1.2	99.07 ± 0.6	99.1 ± 0.52	98.35 ± 1.1
		After Heating cooling cycle			
10.	ZP	-10.86 ± 4.4	-8.29 ± 3.48	-4.98 ± 5.2	-6.85 ± 5.2
11.	GS	15.42 ± 1.27	21.5 ± 2.12	13.67 ± 7.5	17.14 ± 3.5
12.	%T	99.62 ± 0.75	99.35 ± 0.48	98.1 ± 1.35	99.6 ± 0.5

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

4.9.3 Insulin like growth factor-1 mucoadhesive nasal gel:

Mucoadhesive gel was prepared by simple addition of polymers, hydration followed by pH adjustment. The pH of the gel was found to be 5.5 ± 0.1 at 27°C and the viscosity was found to be 45.4 ± 0.2 cp at 25°C.

Graph 4.1 Rheogram of mucoadhesive gel



4.10 DISCUSSION

4.10.1 Clobazam microemulsion and Mucoadhesive microemulsion

Microemulsions of clobazam were successfully prepared using titration method followed by construction of pseudo ternary phase diagram. Based on the solubility study data shown in table 4.1, Capmul MCM was 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. Surfactants, Tween 20 and Acconan CC6 were selected for the study along with cosurfactants like Transcutol 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 and Fig. 4.2). This indicates that increasing surfactant 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. For clobazam, 2 systems were prepared which are System1 [Capmul MCM, Acconan CC6: Tween 20 (3:1), Distilled Water] and System2 [Capmul MCM, Tween 20: Transcutol P (3:1), Distilled Water]. Two microemulsion systems containing clobazam were explored and the results were recorded in Table 4.3 & Table 4.8. In both CZ systems, up to 7.5% v/v of oil was emulsified by 50% of the S_{mix}.

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. Effect of dilution on zeta potential and globule size was studied (Table 4.5) and 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 25°C.

Experimental design (3^2) (Table 4.4 & Table 4.9) was applied in the formulation of microemulsion by varying oil content from 2.5%v/v to 7.5%v/v and S_{mix} from 30%v/v to 50%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 attributed by both the contents of dispersed phase and S_{mix} content (Malmsten Martin 2002). The combined effect of oil content and S_{mix} concentration on zetapotential and globule size were illustrated by the RSM and contour plots (Fig 4.3; Fig. 4.4; Fig. 4.5; Fig. 4.6). The change in the zeta potential with the change in both oil and S_{mix} content was found to follow a systematic pattern which was shown by the multiple regression coefficient values (0.9858 for CZ system1 and 0.9728 for CZ system2). Similarly the influence of oil and S_{mix} content on globule size was found to follow the pattern which was reflected in their multiple regression coefficient values (0. 9760 for CZ system1 and 0. 9896 for CZ system2). Check point experiments (Table 4.6 & Table 4.10) were performed to confirm the utility of polynomial equation and established contour plots in the preparation of microemulsion. In both CZ system1 and CZsytem2, it was found that the globule sizes were below 50nm. The optimized batches of CZ system1 and CZ system2 were selected on the basis of highest zeta potential value with the globules size less than 50nm.

The addition of polymers carbopol and chitosan tends to influence the zetapotential and viscosity of the microemulsions, since these ionic polymers get adsorbed on the interface and influence zeta potential considerably (Fuying et al 2006). It was found that the addition of carbopol to CZME1 tends to raise the zeta potential in negative side and rises with increasing concentration of carbopol. 65% of the external phase volume in the CZME1 limited the addition of carbopol upto 0.5%. Chitosan was found to increase the zeta potential in positive side without significant change in the globule size (Table 4.7). Since the volume of external phase of CZME2 was only 47.5%, the addition of concentrated polymer solution was limited and 0.3%w/v of carbopol in CZME2 and 0.5%w/v of chitosan in CZME2 were prepared. In CZME2, it was found that the addition of carbopol and chitosan to ME tends to increase the zeta potential in negative and positive side respectively which were shown in Table 4.11.

The drug loaded microemulsions and mucoadhesive microemulsions were characterized for their qualitative test, zeta potential, globule size, %transmittance, pH, assay and viscosity and the results were recoded in Table 4.12. 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 nasal cavity secretions and hence would not cause nasal irritation on intranasal administration. Microemulsions were found to possess lower viscosity and exhibit newtonian flow. In the TEM positive image of CZME1, microemulsion appeared dark and the surroundings were bright (Fig 4.15). Some globule sizes were measured as TEM is capable of point to point resolution. The sizes were in agreement with the globule size distribution measured using photon correlation spectroscopy.

The prepared CZ formulations were subjected to nasal toxicity study to evaluate the safety of the ingredients used in the formulation. The optical microscopy images of nasal mucosa treated with CZ formulations were shown in Fig 4.17(C-H). The nasal mucosa treated with isopropyl alcohol (mucociliary toxic agent) showed complete destruction of epithelial layer while nasal mucosa treated with microemulsion and mucoadhesive microemulsion and subsequent washing were found to be intact without much damage of the epithelial layer. Thus prepared formulations were found to be comparatively safe on nasal mucosa than isopropyl alcohol. However further toxicity studies have to be conducted prior to clinical application of the prepared formulations.

In long term stability study, the CZ microemulsions (CZME1 & CZME2) were packed in the borosil screw capped vials and were kept at room temperature (25-35°C) and refrigeration temperature (20°C). During the storage period, micro emulsion systems were assessed for their zeta potential, globule size, physical stability, assay and pH (Table 4.25). 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 CZME1 system were seen. Irrespective of the

storage conditions, the CZME1 system remained stable for 6 months duration. Similar type of results was found for the CZME2 system on storage at similar conditions.

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 empty CZME1 and CZME1 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 4.27). The change in the parameters after accelerated stability conditions was found to be insignificant which clearly indicates that the prepared microemulsion (empty CZME1 & CZME1) systems were thermodynamically stable.

4.10.2 Clopidogrel bisulphate microemulsion and Mucoadhesive microemulsion

Microemulsions of clopidogrel bisulphate were successfully prepared using titration method followed by construction of pseudo ternary phase diagram. Based on the solubility study data shown in Table 4.2, Capmul GMO was 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 20 and Tween 80 were selected for the study along with cosurfactants like Transcutol 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. 4.7 and Fig. 4.8). This indicates that increasing surfactant 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. For clopidogrel bisulphate, three systems were prepared which are System1 [Capmul GMO, Tween 80: Transcutol (2:1), Distilled water], System2 [Capmul GMO, Tween 20: Transcutol (2:1), Acetate buffer (pH 5)] and System3 [Capmul GMO, Tween 20: PEG 200 (3:1), Acetate buffer (pH 5)] Three microemulsion systems containing clopidogrel bisulphate were explored and the results were recorded in Tables 4.14, 4.16 & 4.20. The pH of the microemulsions

prepared with distilled water as an external phase were found to be less than 4.5 which may be irritational on intranasal administration. Since pH of CSME system1 was found to be very low which would cause nasal irritation upon application, various buffers were screened as an external phase in microemulsion (Gallarate et al 1988; Moreno et al 2000). The solubility and stability of clopidogrel bisulphate was studied by preparing a saturated solution of drug in buffers followed by analyzing the supernant for drug content at different time point (Table 4.13). It was found that clopidogrel underwent degradation in acidic pH (below pH4) and had low solubility in acetate buffer pH 5 & pH 6. But pH of CSME systems prepared with acetate buffer pH6 as an external phase was found to be greater than 6. Since phosphate buffers (4-6) have higher solubility than that of corresponding acetate buffers, phosphate buffer pH 5 was taken into consideration for diffusion medium for *in vitro* release studies. Acetate buffer pH 5 was selected as an external phase in CSME systems. The pHs of the microemulsions prepared with acetate buffer (pH5) were found to be with in the nasal secretion range. In both CS systems, up to 7.5% v/v of oil was emulsified by 70% of the S_{mix}.

Experimental design (3^2) (Table 4.17 & 4.21) was applied in the formulation of microemulsion by varying oil content from 2.5%v/v to 7.5%v/v and S_{mix} from 40%v/v to 70%v/v, measuring globule size (GS) and zeta potential (ZP) as the responses. It was found that low surfactant content/ high oil content resulted microemulsions with large size. It was obvious that the zeta potential was attributed by both the contents of dispersed phase and S_{mix} content. ((Malmsten Martin,2002). The combined effect of oil content and S_{mix} concentration on zetapotential and globule size were illustrated by the RSM and contour plots (Fig. 4.9; Fig. 4.10; Fig 4.11; Fig 4.12, Fig 4.13; Fig 4.14). The change in the zeta potential with the change in both oil and S_{mix} content was found to be in a systematic pattern which was shown by the multiple regression coefficient values (0.9934 for CS system2 and 0.9884 for CS system3). Similarly the influence of oil and S_{mix} content on globule size was found to be following a systematic pattern which was reflected in their multiple regression coefficient values (0.9834 for CS system2 and 0.9899 for CS system3). Check point experiments (Tables 4.18 for CSME2 and Table 4.22 for CSME3) were performed to confirm the utility of polynomial equation and established contour plots in the preparation of microemulsion. It was found that the globule sizes were below 50nm in

both CS system2 and CS sytem3. The optimized batches of CS system2 and CS system3 were selected on the basis of highest zeta potential value with the globules size less than 50nm.

The addition of polymers carbopol and chitosan tends to influence the zetapotential and viscosity of the microemulsions, since these ionic polymers get adsorbed on the interface and influence zeta potential considerably (Fuying et al 2006). It was found that the addition of carbopol to CSME2 tends to raise the zeta potential in negative side and rises with increasing concentration of carbopol. Since the external phase of CSME2 was only 27.5%v/v, the addition of concentrated polymer solution was limited and 0.25%w/v of carbopol CSME2 and 0.75%w/v of chitosan CSME2 were prepared Table 4.19. Higher volume of external phase in CSME3 (57.5%v/v) made possible for the formulation of 0.5%w/v carbopol CSME3 and 1%w/v chitosan CSME3 (Table 4.23). Under these studies it was observed that the addition of carbopol and chitosan to ME not only increase the viscosity of the formulations but also tends to increase the zeta potential in negative and positive side respectively without significant change in the globule size (Table 4.19 & Table 4.23).

The drug loaded microemulsions and mucoadhesive microemulsions were characterized for their qualitative test, zeta potential, globule size, %transmittance, pH, assay and viscosity and the results were recoded in Table 4.24. 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 were of o/w type microemulsion. The pH of the formulations were adjusted with the selection of external phase and made to be within the range of nasal cavity secretions. Microemulsions were found to possess lower viscosity and exhibit newtonian flow. In the TEM positive image of CSME3, microemulsion appeared dark and the surroundings were bright (Fig. 4.16). Some globule sizes were measured as TEM is capable of point to point resolution. The sizes were in agreement with the globule size distribution measured using photon correlation spectroscopy.

The prepared CS formulations were subjected to nasal toxicity study to evaluate the safety of the ingredients used in the formulation. The optical microscopy images of nasal mucosa treated with CS formulations were shown in Fig. 4.17(I-N).

was evaluated for pH, viscosity, IGF-1 content and nasal toxicity study. The pH of the gel was found to be within the nasal secretion range and viscosity was found to be 45.4 ± 0.2 cp at 25°C and the rheogram (Graph 4.1) shows peudoplastic flow with positive thixotropy.

The prepared gel was subjected to nasal toxicity study to evaluate the safety of the ingredients used in the formulation. The optical microscopy images of nasal mucosa treated with gel was shown in Fig 4.17(O). The nasal mucosa treated with isopropyl alcohol (mucociliary toxic agent) showed complete destruction of epithelial layer while nasal mucosa treated with gel and subsequent washing was found to be intact. Thus prepared gel formulation was found to be comparatively safe on nasal mucosa than isopropyl alcohol. However further toxicity studies have to be conducted prior to clinical application of the prepared formulations.

Microemulsions and mucoadhesive microemulsions containing clobazam and clopidogrel bisulphate were successfully prepared and characterized. *The promising and stable formulations listed in Table 4.12 and Table 4.24 were further taken up for in vitro diffusion studies. The prepared mucoadhesive gel was taken up for pharmacokinetic study.*

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