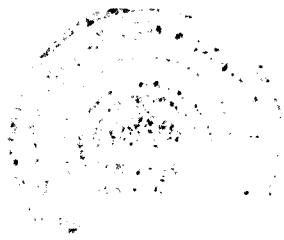
CHAPTER 5

PREPARATION, OPTIMIZATION AND CHARACTERIZATION OF MICROEMULSION AND NANOEMULSION



5 PREPARATION, OPTIMIZATION AND CHARACTERIZATION OF MICROEMULSION AND NANOEMULSION

5.1 Introduction

Microemulsions (MEs) are thermodynamically stable system that is 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. This technique is mainly titration method followed by pseudo ternary phase diagram construction. (Lawrence MJ and Rees GD, 2000) Regardless of the type of ME systems, ME 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 ME are thermodynamically stable systems, they undergo spontaneous formation, facilitated by micelle formation without inputs 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 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, 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 MJ and Rees GD, 2000).

MEs are equilibrium systems (i.e. thermodynamically stable), while nanoemulsions (NEs) are non-equilibrium systems with a spontaneous tendency to separate into the constituent phases. Nevertheless, NEs may possess a relatively high kinetic stability, even for several years (Solans C et al., 2003). NEs can be obtaind by high shear methods, ultrasonication and condensation method and globule size (GS) of NEs is in nanometer range. Evidently, the preparation method influences emulsion properties (e.g. droplet size, stability, etc.), but the nature of the final dispersion (the constituent phases) is the same whether the method of

preparation uses high shear (external energy, dispersion methods) or the chemical energy stored in the system (condensation methods).

Resently, Ultrasonication method has been explored for preparation of Nanoemulsion. (Kentish S et al., 2008) Studies to date comparing ultrasonic emulsification with rotor–stator dispersing have found ultrasound to be competitive or even superior in terms of droplet size and energy efficiency (Abismail B et al., 1999, Tadros T et al., 2004). Microfluidization has been found to be more efficient than ultrasound, but less practicable with respect to production cost, equipment contamination and aseptic processing (Abismail B et al., 1999). Comparing mechanical agitation to ultrasound at low frequency, Tadros T et al. (2004) found that for a given desired diameter, the surfactant amount required was reduced, energy consumption (through heat loss) was lower and the ultrasonic emulsions were less polydisperse and more stable.Ultrasonic emulsification is believed to occur through two mechanisms. Firstly, the application of an acoustic field produces interfacial waves which become unstable, eventually resulting in the eruption of the oil phase into the water medium in the form of droplets. (Li MK and Fogler HS, 1978a) Secondly, the application of low frequency ultrasound causes acoustic cavitation, that is, the formation and subsequent collapse of microbubbles by the pressure fluctuations of a simple sound wave. Each bubble collapse (an implosion on a microscopic scale) event causes extreme levels of highly localized turbulence. The turbulent micro-implosions act as a very effective method of breaking up primary droplets of dispersed oil into droplets of sub-micron size. (Li MK and Fogler HS, 1978b)

Emulsification by ultrasonication was selected for the preparation of lipid NE in this investigation due to non-tediousness and feasiblility at lab scale compared to other techniques. Lipid NE of drug prepared using this technique constituted of one or combination of emulsifiers. Prepared lipid NE was characterized for globule size (GS) and size distribution (PDI), zeta potential (ZP), pH, viscosity, % transmission (%T) and total drug content. Optimization was carried out by selection of various formulations and process variables which would yields well-defined, reproducible lipid NE of desired droplet size.

5.2 Methods

5.2.1 Solubility determination

The solubility of the drug in oils and surfactants is most important, as the ability of emulsion to maintain the drug in solubilized form is greatly influenced by solubility of the drug in oil phase. Solubility of drugs, Tramadol (TMD) and Lamotrigine (LTG), was determined in different oils and surfactants. An excess amount of drug was added to 2ml of selected oils in 5ml stopper vials and then it was kept under moderate mechanical shaking using isothermal shaker (VORCO) for 24h to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 6000 rpm for 10 min. The supernatant was taken and filtered through a 0.45-µm membrane filter. An aliquot of the supernatant was diluted with solvent described in analytical section respectively and drug content was assayed by UV spectroscopy. Solubility study of TMD and LTG is recorded in Table 5.1 and 5.2 respectively.

5.2.2 Preparation of drug solutions

Drug solutions, TMD solution (TS) and LTG solution (LS) were prepared for the comparative evaluation of ME based systems. From the solubility study results, TS (9.75 mg/ml) was prepared by dissolving TMD in a mixture of propylene glycol (15%), PEG-400 (7.5%), Twen-80 (0.5%) and water (77%). LS (6.50 mg/ml) was prepared by dissolving LTG in a mixture of propylene glycol (10%), PEG-400 (5%) and water (85%). pH of both drug solution was adjusted to 5.5

5.2.3 Preparation and optimization of Microemulsion

Based on the solubility study, for TMD oil (IPM), surfactant (Labrasol) and cosurfactant (Tween-20) were selected and different ratios of surfactant and cosurfactant (1:1 to 3:1) were studied in the phase diagram construction. From the solubility study, oil (Capmul MCM), surfactant (Tween-20) and cosurfactant (Transcutol) for LTG were selected and different ratios of surfactant and cosurfactant (1:1 to 4:1) were studied in the phase diagram construction.

5.2.3.1 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 dropwise to each oil- S_{mix} mixture under vigorous stirring. After equilibrium, the samples were visually checked for clarity of ME. 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 ME region. The area of the monophasic region was used as a tool for the selection of suitable surfactant and co surfactant mixture.

5.2.3.2 Preparation of Microemulsion

Based on the phase diagram, the optimum S_{mix} ratio was selected and the drug loaded ME were prepared by dissolving the required quantity of drug in the oil- S_{mix} mixture to obtain loading of 12.19 mg/ml and 8.13 mg/ml for TMD and LTG respectively. Water was added to drug loaded internal phase in dropwise manner under continuous stirring.

5.2.3.3 Optimization of Microemulsion

Formulation of TMD containing different % of oil (2.5%, 5% and 7.5% v/v), surfactant-cosurfactant mixture (20%, 30%, 40% and 50% v/v) and water was tabulated in Table 5.3. LTG MEs were prepared by using different % of oil (3%, 6% and 9% v/v), S_{mix} (20%, 30%, 40% and 50% v/v) and water and formulations were tabulated in Table 5.4. MEs were evaluated for GS, ZP, %T and dilution characteristic. Consider the amount and solubility of drug to be incorporated in the ME for the selection of formulation. The final composition of ME was optimized based on GS, ZP, %T and dilution characteristics.

5.2.4 Preparation and optimization of Nanoemulsion

Based up on solubility study oil used in NE formulation was selected. Oil concentration in NE formulation was decided by considering drug loading in formulation and upon solubility of drug in oil provided it form physically stable NE system. However, oil concentration upto 20% are used in marketed NE. (Celepid fat emulsion) Suitable surfactant was selected based upon literature (Singh KK and Vingkar SK, 2008). Drug loading in formulation was decided from dose to be administered. TNE was consisting of Isopropyl myristate (IPM) (17.5%), soya lecithin, poloxamer and TMD (9.75 mg/ml) while, LNE containing Capmul MCM (15%), soya lecithin, poloxamer and LTG (6.5 mg/ml).

5.2.4.1 Preparation of Nanoemulsion

Lipid NE was formulated by sonication emulsification method. (Tadros T et al., 2004) For the preparation of o/w NE, aqueous and oil phases were separately prepared. The aqueous phase consisted of water and poloxamer. The oil phase consisted of oil, surfactant and drug. The two phases were heated separately to 50–60 °C. Then oil phase was gradually added to the aqueous phase with constant stirring on a magnetic stirrer at this temperature, to get coarse emulsion. The resultant coarse emulsion was sonicated for 10 min using the bench type probe sonicator (LABSONIC®M, Sartorius, Mumbai, India). The probe sonicator was adjusted at 21% amplitude and 50% duty cycle. NE were adjusted to the appropriate pH and filtered through a 0.45 μ membrane filter.

5.2.4.2 Optimization of Nanoemulsion

Surfactant concentration is major formulation variable affecting NE characteristics. (Gutierrez JM et al., 2008) Surfactant to be used, concentration of drug and oil used was decided as describe earlier. Concentration of surfactant has to be optimized.

Optimization of surfactant concentration

TNE and LNE formulation containing varying % of surfactant (Soya lacithin) for internal phase were prepared by keeping other ingredients of formulation constant. Formulations were evaluated for GS and PDI by Malvern zetasizer. GS and PDI of formulation were also measured after 15 days. Optimization of surfactant concentration was carried out based on initial and 15th day data for GS and PDI. Optimization of Soya lecithin concentration for TNE and LNE is shown in Table 5.5 and 5.7 respectively.

TNE and LNE formulation containing varying % of non-ionic surfactant (Pluronic F68) for continuous phase were prepared by keeping other ingredients of formulation constant. Formulations were evaluated for GS and PDI by Malvern zetasizer. GS and PDI of formulation were also measured after 15 days. Optimization of surfactant concentration was carried out based on initial and 15th day data for GS and PDI. Optimization of poloxamer concentration for TNE and LNE is shown in Table 5.6 and 5.8 respectively.

5.2.5 Characterization of Microemulsion and Nanoemulsion

The characterization of ME and NE is essential before proceeding for the *in vivo* studies. The characterization is performed for predicting the reproducible characteristics of the prepared formulation. ME and NE were characterized for GS, ZP, Assay, *in vitro* drug release (drug

diffusion), surface morphology and viscosity. Various techniques for characterization of ME and NE include:

- Photon correlation spectroscopy (PCS) based on the dynamic light scattering (DLS) for GS and its distribution.
- The surface characteristic like charge is examined by measurement of ZP, surface morphology by transmission electron microscopy (TEM).
- Amount of drug present in ME and NE is determined as assay.
- The *in vitro* release of the drug from the ME and NE influences the *in vivo* pharmacokinetic and pharmacodynamic behavior and is estimated by spectrophotometric method.
- Viscosity of ME and NE affect delivery method and distribution to various organs. Viscosity of ME and NE is determined by Brookfield Viscometer.

5.2.5.1 Appearance

Appearance of ME and NE of TMD and LTG was evaluated against white and black background and recorded in Table 5.9.

5.2.5.2 Globule size and Zeta potential determination

The GS determination (Kaler EW and Prager SK, 1982; Roland I et al., 2003) of TMD and LTG loaded ME and NE were determined using photon correlation spectroscopy (PCS) with in-built Zetasizer (model: Nano ZS, Malvern instruments, UK) at 633 nm.

Measurement conditions for GS were optimized by measuring GS for the dispersions of different dilutions. The dilution of the ME and NE in water 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.

Malvern Zetasizer Nano ZS was used to measure the ZP 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 ZP by in-built software based on Helmholtz-Smoluchowski equation. Measurements were performed using small volume disposable zeta cell. The observations for ME, NE of TMD and LTG are tabulated in Table 5.9.

5.2.5.3 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 S and Faiyaz S, 2007). The images were taken Tecnai200 with CCD camera operating at 200kV (Philips Instruments, Holland) and capable of point to point resolution . ME and NE were diluted in de-ionized water (1 in 10 dilution). To measure the morphology and size distribution, a drop of sample was placed onto a 300-MEh copper grid coated with carbon. Approximately 2 min after deposition, the grid was tapped with filter paper to remove surface water and air-dried. Negative staining was performed using a droplet of 0.5% w/v phosphotungstic acid. Transmisssion electron microscopy was performed using Morgagni 268, Philips (Netherlands) transmission electron microscope. The TEM images for ME, NE for TMD and LTG are shown in Fig. 5.3 and 5.4 respectively.

5.2.5.4 Estimation of drug in Microemulsion and Nanoemulsion

0.1 ml of ME and NE was sufficiently diluted with methanol. Absorbance was measured by UV-Visible spectrophotometer. Estimation of drug in ME and NE was determined as per the method described in the Analytical section and the results were recorded in tables 5.9.

5.2.5.5 pH Determination

The pH of ME and NE of TMD and LTG was measured by digital pH meter at 25° C ± 1°C. The pH was recorded in triplicate and data were shown in Table 5.9. pH meter was calibrated using buffer solutions prior to use.

5.2.5.6 Viscosity Determination

Viscosity of the formulations was determined using Brookfield cone and plate Rheometer (Model LVDV III) using CPE spindle at the rotational speed of 5rpm, shear rate of 10 at room temperature and the results were recorded in Table 5.9.

5.2.5.7 Transmittance

The percentage transmittance of ME was checked against distilled water using UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan) at 630 nm. Results are given in Table 5.9.

5.2.5.8 *In vitro* drug diffusion study

In vitro diffusion of formulations is a valuable tool to predict the behaviour of a particular formulation with respect to drug transport across the membrane. According to Gemmell DH

and Morrison JC (1957), *in vitro* models may have limitations in terms of prediction of drug transport across the mucosal membrane nevertheless: under the testing conditions *in vitro* studies can be helpful to access the relative drug transport behavior across the mucosa. Various parameters pertaining to formulations such as flux, partition coefficient and diffusion coefficient can be derived using *in vitro* evaluation techniques. In present study, all the test formulations were accessed for *in vitro* diffusion across the sheep nasal mucosa. The study was performed in triplicate and the parameters were calculated.

A. Percent drug diffused

The percent drug diffused across the sheep nasal mucosa at predetermined sampling time interval using formula mentioned below.

$$\% \text{ Drug diffused} = \frac{\text{Amount of drug in receptor compartment at time t}}{\text{Amount of drug loaded in the donor compartment}} \times 100$$

B. Kinetics of release

In order to investigate the mechanism of drug release from the formulation, the release rates were integrated into each of the following equation and the regression coefficient was calculated.

i. Zero order equation

$Q = K_0 t$

Where, Q is amount of drug released at time t (h) and K_0 is zero order release rate constant

ii. First order equation

$Q = Q_0 e^{-K_1 t}$

Where Q, is amount of drug released at time t (h) and K_1 is first order release rate constant.

iii. Higuchi's equation

$Q = K_H x \sqrt{t}$

Where, Q is amount of drug released at time t (h) and K_H is zero order release rate constant.

iv Hixson-crowell cube root law

$Q_0^{1/3} - Q_t^{1/3} = K_{HC} t$

Where, Q_0 is initial amount of the drug in the formulation, Q_t is amount of the drug released at time t (h) and K_{HC} is Hixson-Crowell rate constant.

v. Korsmeyer-peppas equation

$$\frac{M_t}{M_\infty} = Kt^n$$

Where M_t - amount of the released drug at time t (h), M_∞ is total amount of drug released after an infinite time, K is diffusional characteristic constant of drug/polymer system and n is exponent that characterizes the mechanism of drug release.

The order of release was determined by performing the regression over the mean values of percent drug diffused vs. time (for zero order), log percent drug diffused vs. time (for first order), percent drug diffused vs. square root of time (for higuchi order), difference of cube root of percent total drug and percent drug released vs. time (Hixson-crowell cube root law) and log cumulative percentage of drug released vs. log time (Korsmeyer-peppas equation).

C. Flux

The skin flux can be experimentally determined from the following equation (Lee J et al., 2005)

$$J = (dQ/dt)/A$$

Where, J is the steady-state flux (μg/cm^2/h), A is the diffusion area of skin tissue (cm^2) through which drug permeation takes place, and dQ/dt is the amount of drug passing through the skin per unit time at a steady-state (μg/h). The cumulative amount of drug permeating through the membrane was plotted as a function of time.

D. Diffusion coefficient

The diffusion coefficient of the drug was calculated using the following equation (Alton ME, 2007)

$$D = J \times h/C_0$$

Where, J is Flux, C_0 is drug concentration in donor compartment and h is thickness of the membrane.

Franz diffusion cell

The *in vitro* diffusion studies were carried out using Franz diffusion cell. This cell consists of a hollow glass tube in the center having diameter of 10 mm. The cell has two compartments viz. i) donor compartment and ii) receptor compartment. The donor compartment is used for holding the test formulation while the receptor compartment holds the respective diffusion media. The hydrodynamic characteristics of the franz diffusion cell was established using benzoic acid disc method (Chein YW, Valia KH, 1984)

Preparation of membrane

The freshly excised sheep nasal mucosa, except septum part was collected from the slaughter and was kept in PBS pH 6.4 for 15 min to equilibrate. The superior nasal conche was identified and separated from the nasal membrane and made free from adhered tissues. Selective samples of tissues of 0.2 mm thickness were taken for the studies. The excised nasal membrane was then mounted on franz diffusion cell. The tissue was stabilized using phosphate buffer pH 6.4 in both the compartments and allowed to stir for 15 min on a magnetic stirrer. After 15 min, solution from both the compartments was removed and the diffusion media was filled in the acceptor compartment. The mounting of the nasal mucosa was done using glue at the brim of the donor compartment to avoid the leakage of the test sample and supported with rubber bands crossover the cell. The temperature of the receiver chamber containing diffusion media was controlled at 37° ± 1° C under continuous stirring with teflon coated magnetic bar at constant rate, in such a way that the nasal membrane surface just flushes the diffusion media.

Diffusion study

In-vitro drug diffusion study was performed using Franz diffusion cell with a diameter of 10 mm and mucosa thickness (height) 0.2 mm (Willimann H et al., 1992). PBS pH 5 + 2% Tween 80 and PBS pH5 + 1% SLS was used as a diffusion media for diffusion study of TMD and LTG loaded MEs and NEs respectively. 0.1 ml of ME or 0.12 ml of NE was placed in the donor compartment along with 0.1 ml of diffusion media. Recipient compartment containing 12 ml of medium was stirred with Teflon coated magnetic bead. Samples from the receptor compartment were withdrawn at predetermined time intervals and analyzed by UV spectrophotometry method as discussed in chapter 3, section 3.3.1.3. and 3.3.2.3. Each sample removed was replaced by an equal volume diffusion media. Study was carried for a period of 6 h, during which the drug in receiver chamber (µg/ml) across the sheep nasal membrane calculated at each sampling point. The diffusion study of drug was performed in a similar manner for drug solution. The formulations were studied in triplicate for diffusion studies and the mean cumulative values for % drug release of TMD and LTG were shown in Fig. 5.5 and 5.6 respectively. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, Higuchi's equation, first order equations, Hixson-crowell cube root law, Korsmeyer-peppas equation and recorded in Table 5.10 and 5.11. The diffusion coefficients and flux were determined and tabulated in Table 5.12.

5.2.6 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. Mucosa were treated with 0.5 ml of PBS pH 6.4, isopropyl alcohol , MEs (TME, LME) and NEs (TNE, LNE) for 1 h as well as 2 h. 2 h samples were treated PBS pH 6.4 for 15 min after 1h of treatment followed by additional 1h treatment of respective formulations. After 1or 2 h the mucosa were rinsed with PBS pH 6.4 and carried to the pathological laboratory in 10% formalin for the preparation of pathological slides. Nasal mucosa was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Paraffin sections were cut on glass slides and stained with hematoxylin and eosin. The sheep nasal mucosa treated with PBS pH 6.4 and isopropyl alcohol were taken as negative and positive control respectively. Sections were examined under a light microscope, to detect any damage to the mucosa during *in vitro* permeation by a pathologist blinded to the study (Majithiya R et al., 2006). Figures 5.6 show the histopathological images of nasal mucosa for phosphate buffer (pH 6.4), IPA and all drug loaded MEs and NEs formulations after 1 h and 3 h of treatment.

5.3 Results and Discussion

5.3.1 Solubility study of Tramadol and Lamotrigine

Table 5.1 Solubility of TMD in oils and surfactants

Sr. No.	Excipients	Solubility (mg/ml)
1	Aconon CC6	6.47 ± 0.52
2	Aconon MC8	10.33 ± 0.83
3	Capmul MCM	6.45 ± 0.44
4	Capryol 90	24.58 ± 1.35
5	Capryol PGMC	32.21 ± 2.82
6	Captex 355 EP/NF	33.98 ± 1.98
7	Captex 500	26.44 ± 2.16
8	Crillet	8.67 ± 0.63
9	Ethyl Oleate	< 2
10	IPM	60.58 ± 4.54
11	Labrafac	5.72 ± 0.43
12	Labrafac Lipophile WL1349	10.41 ± 0.76
13	Labrafac PG	8.23 ± 0.81
14	Labrasol	87.73 ± 4.73
15	Lauroglycol FCC	14.47 ± 0.22
16	Miglyol	42.68 ± 2.36
17	Oleic acid	< 2
18	Peanut oil	4.34 ± 0.21
19	PEG 400	107.26 ± 6.79
20	Soyabean oil	< 2
21	Transcutol	40.82 ± 3.21
22	Tween 20	53.43 ± 2.86
23	Tween-80	27.44 ± 1.20

** Values are represented as mean ± SD, n=3*

Table 5.2 Solubility of LTG in oils and surfactants

Sr. No.	Excipients	Solubility (mg/ml)*
1	Aconon CC6	30.93 ± 1.79
2	Aconon MC-8	38.22 ± 2.94
3	Capmul MCM	52.63 ± 2.21
4	Caprol 100-100	< 2
5	Capryol 90	16.59 ± 0.88
6	Capryol PGMC	11.30 ± 1.21
7	Captex 500	9.26 ± 0.73
8	Cromophor EL	< 2
9	Cromophor RH-40	< 2
10	Ethyl oleate	< 2
11	Labrafel M 1944CS	10.44 ± 0.67
12	Labrasol	47.78 ± 3.52
13	Lauroglycol FCC	< 2
14	Lauroglycol-90	< 2
15	Miglyol	< 2
16	Oleic acid	3.76 ± 0.28
17	Peanut oil	< 2
18	Peceol	18.11 ± 1.92
19	PEG-400	124.31 ± 5.37
20	Sesame oil	4.72 ± 0.39
21	Soyabean oil	3.54 ± 0.42
22	Transcutol	135.93 ± 6.58
23	Tween-20	59.26 ± 2.88
24	Tween-80	27.96 ± 1.27

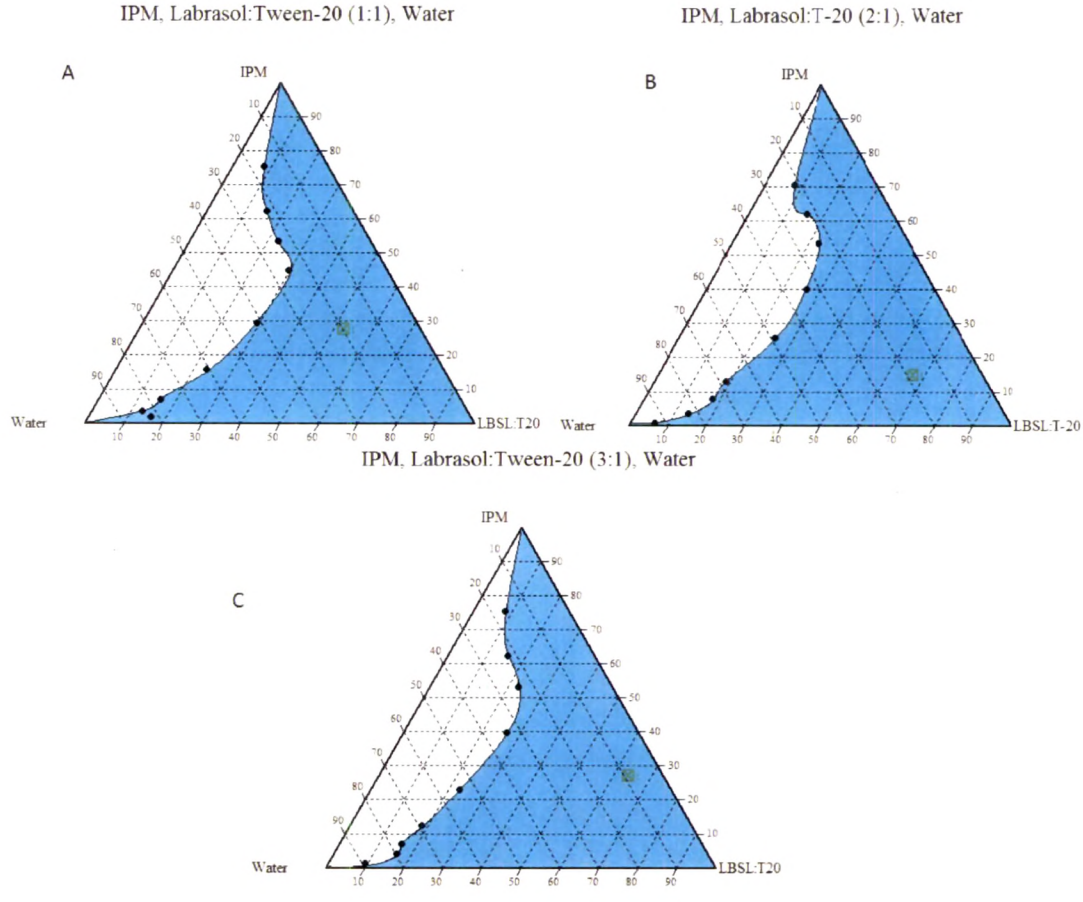
* Values are represented as mean ± SD, n=3

Solubility study results of TMD and LTG shown in Table 5.1 and 5.2 respectively. Results of solubility study of TMD revels that Labrasol, Miglyol, IPM, PEG-400, Transcutol and Tween-20 have solubility more than 40mg/ml. Results of solubility study of LTG revels that Capmul MCM, Labrasol, Transcutol, PEG-400, and Tween-20 have solubility more than 40 mg/ml.

5.3.2 Formulations of Tramadol and Lamotrigine Microemulsions

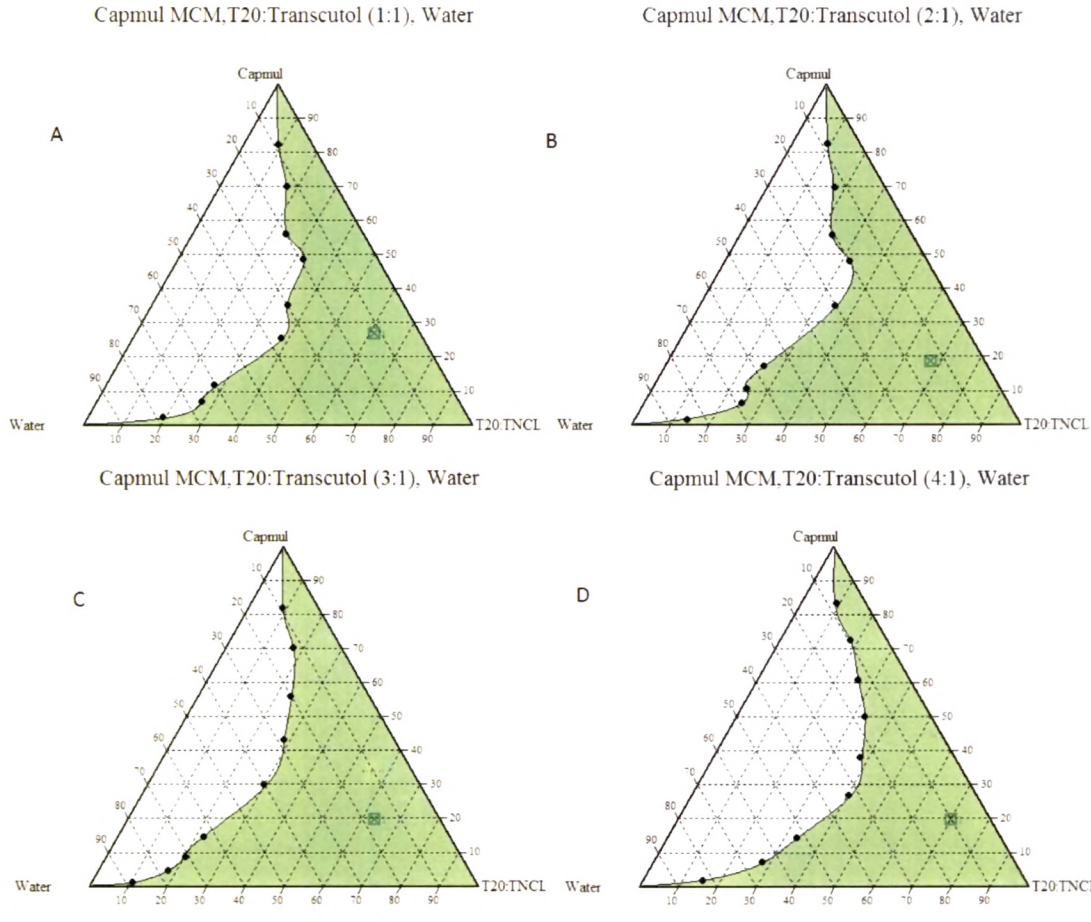
Phase diagram

Figure 5.1: Phase diagram of TME with varying surfactant: cosurfactant ratio (A) 1:1 (B) 2:1 (C) 3:1



IPM and Capmul MCM was selected as an internal phase for the preparation of TME and LME respectively. 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 (Phil Mc Williams and Graham Payne, 2001) and hence, use of non-ionic surfactant for pharmaceutical ME formulation is gradually increasing. For TME and LME Surfactant/cosurfactant Labrasol/Tween-20 and Tween-20/Transcutol were selected for the formulation of TME and LME respectively.

Figure 5.2: Phase diagram of LME with varying surfactant: cosurfactant ratio (A) 1:1 (B) 2:1 (C) 3:1 (D) 4:1



ME of TMD and LTG were successfully prepared using titration method followed by construction of pseudo ternary phase diagram. Different ratios of surfactant:cosurfactant for TME (1:1 to 4:1) and for LME (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 ME region increased toward water-oil axis. This indicates that increasing surfactant concentration the maximum amount of oil can be solubilised/ emulsified. However surfactant concentration should be used at minimum. This was earlier reported by Lianli Li et al. (2002) and Zhang Q et al. (2004).The increased oil content may provide opportunity for the solubilisation of the drug.

Table 5.3 Formulation of TME

Sr. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	GS* (nm)	ZP* (mV)	%T	Physical evaluation
1	TME1	2.5	20	75	--	--	--	Not soluble
2	TME2	2.5	30	65	NA	NA	NA	TOD
3	TME3	2.5	40	55	NA	NA	NA	TOD
4	TME4	2.5	50	45	25.44 ± 3.87	-5.54 ± 0.74	98.45 ± 0.56	Clear
5	TME5	5	20	72.5	NA	NA	NA	TOD
6	**TME6**	**5**	30	**62.5**	**16.69 ± 3.01**	**-8.97 ± 0.53**	**99.09 ± 0.42**	Clear
7	TME7	5	40	52.5	15.32 ± 2.92	-7.64 ± 0.67	98.78 ± 1.43	Clear
8	TME8	5	50	4.5	14.75 ± 1.86	-6.82 ± 1.05	98.85 ± 0.84	Clear
9	TME9	7.5	20	70	--	--	--	Not formed
10	TME10	7.5	30	60	--	--	--	Not formed
11	TME11	7.5	40	50	--	--	--	Not formed
12	TME12	7.5	50	40	NA	NA	NA	TOD

** Values are represented as mean ± SD, n=3; TOD - Turbid on dilution; NA - Not analyzed*

Table 5.4 Formulation of LME

Sr. No.	Formulation	% v/v Oil	% v/v S_{mix}	% v/v Water	GS* (nm)	ZP* (mV)	%T	Physical evaluation
1	LME1	3	20	77	--	--	--	Not formed
2	LME2	3	30	67	NA	NA	NA	TOD
3	LME3	3	40	57	22.16 ± 3.21	-8.59 ± 1.34	97.65 ± 0.98	Clear
4	LME4	3	50	47	18.39 ± 1.56	-7.84 ± 0.67	98.83 ± 1.05	Clear
5	LME5	6	20	74	--	--	--	Not formed
6	LME6	6	30	64	NA	NA	NA	TOD
7	**LME7**	**6**	**40**	**54**	**14.25 ± 2.73**	**-9.42 ± 0.82**	**99.74 ± 0.53**	**Clear**
8	LME8	6	50	43	13.88 ± 1.84	-8.14 ± 0.73	98.68 ± 0.75	Clear
9	LME9	9	20	71	--	--	--	Not formed
10	LME10	9	30	61	--	--	--	Not formed
11	LME11	9	40	51	--	--	--	Not formed
12	LME12	9	50	41	NA	NA	NA	TOD

** Values are represented as mean ± SD, n=3; TOD - Turbid on dilution; NA – Not analyzed*

Table 5.3 represent the formulation of TME containing varying oil content from 2.5%v/v to 7.5%v/v and S_{mix} from 20%v/v to 50%v/v, measuring GS and ZP as the responses. Formulation of LME containing varying oil content from 3%v/v to 9%v/v and S_{mix} from 20%v/v to 50%v/v, measuring GS and ZP as the responses shows in Table 5.4. In TME up to 5% v/v of oil was emulsified by 50% of the S_{mix} while, in LME up to 6% v/v of oil was emulsified by 50% of S_{mix}.

Drug was not soluble in batch TME1 because of low % of oil as well as S_{mix}. Batch TME2, TME3 was became turbid on dilution because in both batches % of oil was low. Batch TME6 also became turbid on dilution because this batch contain low % of S_{mix} and TME12 was found to be turbid on dilution because of excess oil content by which it lied on boundary of ME region of phase diagram. Batches TME5, TME9, TME10 and TME11 were not formed because it contains low % of S_{mix} with respect to its oil content. Batches TME4, TME6, TME7 and TME8 were found physically stable against dilution thus GS and ZP of these batches were evaluated. Batch TME4 showed higher GS and ZP value compare to batch no. 6, 7 and 8. TME7 and TME8 have GS less than GS of TME6 but when comparing ZP of these 3 batches it shows that ZP of both TME7 and TME8 shows higher ZP towards zero. Thus, TME6 was selected as optimized batch considering highest amplitude of ZP. Higher ZP in this case, towards zero represents more aggregation of globules.

Batch LME1 was not formed because of low % content of oil and S_{mix}. Batches LME5, LME9, LME10 and LME11 were also not formed because it contains low % of S_{mix} with respect to its oil content. LME2 contains low % of oil so it became turbid on dilution. LME6 became turbid on dilution because it contains low % of S_{mix}. LME12 also became turbid on dilution because it contains high % of oil which lied on boundary of ME region of phase diagram. Batch no. 3, 4, 7 and 8 of LME was found physically stable against dilution so these batches were evaluated for GS and ZP. Results of evaluation shows that LME3 and LME4 have higher value of GS compare to LME7 and LME8. LME8 have low GS than LME7 but higher ZP value means toward zero than LME7. Thus, LME7 was taken as optimized batch and selected as final formulation of LME for further study.

5.3.3 Formulations of Tramadol and Lamotrigine Nanoemulsions

Lipid NE of drug was prepared by ultrasonication method, which has been successfully used to reduce oil droplet size of NE to below 250nm.

5.3.3.1 Optimization of Tramadol Nanoemulsion

TNE composed of IPM as oil, Soya lecithin as Internal phase surfactant, poloxamer as continuous phase surfactant and Water as continuous phase.

In this formulation surfactant concentration is to be optimized by keeping other components constant. Prepared formulation containing varying Soya lacithin concentrations (1%w/v, 1.5%w/v, 2%w/v & 2.5%w/v) and keeping IPM 17.5%v/v, poloxamer 1.5% and water q.s. as constant ingredients were evaluated.

Optimization of poloxamer concentration was done by evaluating formulation containing varying concentrations of poloxamer (1%w/v, 1.5%w/v, 2%w/v & 2.5%w/v) and keeping IPM 17.5%, Soya lecithin 2%, water q.s. as constant ingredients.

Table 5.5 Optimization of Soya lecithin concentration in TNE

Batch	Conc. of Soya lecithin (w/v)	Initial		After 15 days	
		GS (nm)	PDI	GS (nm)	PDI
TNE1	1%	263.7 ± 6.3	0.204	424.6 ± 7.9	0.294
TNE2	1.5%	184.3 ± 5.4	0.163	276.5 ± 3.9	0.195
TNE3	**2%**	**147.4 ± 4.6**	**0.128**	**154.0 ± 5.1**	**0.152**
TNE4	2.5%	158.5 ± 5.7	0.126	166.2 ± 4.8	0.153

Values are represented mean± SD, n=3

Table 5.6 Optimization of poloxamer concentration in TNE

Batch	Conc. of poloxamer (w/v)	Initial		After 15 days	
		GS (nm)	PDI	GS (nm)	PDI
TNE5	1%	177.9 ± 5.4	0.161	337 ± 6.8	0.275
TNE6	1.5%	125.4 ± 3.8	0.118	205.2 ± 6.6	0.189
TNE7	**2%**	**136.2 ± 4.3**	**0.123**	**142.9 ± 3.5**	**0.146**
TNE8	2.5%	143.5 ± 4.6	0.129	158.4 ± 4.4	0.162

Values are represented mean± SD, n=3

Optimization of Soya lecithin concentration in TNE formulation was done by observing the effect of various concentrations of Soya lecithin (emulsifier) on GS and PDI of formulation.

PDI represents uniformity of particle size distribution. Higher value of PDI indicates nonuniform distribution of particle size. PDI less than 0.2 is desirable. PDI values lower than 0.25 indicate a close size distribution providing good stability of nanoemulsions due to the reduced Ostwald ripening (Yilmaz E and Borchert HH, 2005).

As concentration of Soyalacithin was increased up to 2%w/v, the GS of formulation was decreased. Further increasing Soya lecithin conc. (2.5%), it shows increasing GS. TNE3 shows least GS among TNE1, TNE2, TNE3 and TNE4. All these batches were evaluated after 15 days for GS and PDI. Results shows increased value of GS and PDI as compare to initial. However, increment in size was nonsignificant ($P>0.05$) for TNE3 and TNE4. After 15 days GS value of batches 1, 2, 3 and 4 were compared, it shows that TNE3 have low GS than others. Thus, TNE3 was taken as optimized batch and 2% concentration of Soya lacithin was selected and was taken in final formulation of TNE.

Optimization of poloxamer concentration in TNE formulation was done by observing the effect of varying concentrations (1, 1.5, 2 and 2.5%) of poloxamer on GS and PDI of formulation. Results show that TNE6 have least GS and PDI compare to TNE5, TNE7 and TNE8. These all batches were evaluated for GS and PDI after 15 days also. Results after 15 days indicate that TNE7 have less GS and PDI value compare to remaining batches. Thus, batch TNE7 containing poloxamer concentration 2% was optimized and thus % of poloxamer in final formulation of TNE was taken 2%.

Thus final formulation of TNE composed of IPM (17.5%), TMD (9.75mg/ml), Soyalacithin (2%), poloxamer (2%) and water (q.s.).

5.3.3.2 Optimization of Lamotrigine Nanoemulsion

LNE composed Capmul MCM as oil, Soya lecithin as Internal phase surfactant, poloxamer as continuous phase surfactant and Water as continuous phase.

In this formulation surfactant concentration is to be optimized by keeping other components constant. Prepared formulation containing varying Soya lacithin concentratios (1%w/v, 1.25%w/v, 1.5%w/v & 2%w/v) and keeping Capmul MCm 15%, poloxamer 1.5%, water q.s. as constant ingredients were evaluated.

Optimization of poloxamer concentration was done by evaluating formulation containing varying concentrations of poloxamer (1%w/v, 1.5%w/v, 2%w/v & 2.5%w/v) and keeping Capmul MCM 15%, Soya lecithin 1.5%, water q.s. as constant ingredients.

Table 5.7 Optimization of Soya lecithin concentration in LNE

Batch	Conc. of Soya lecithin (w/v)	Initial		After 15 days	
		GS (nm)	PDI	GS (nm)	PDI
LNE1	1%	274.6 ± 6.8	0.199	505.4 ± 7.3	0.278
LNE2	1.25%	179.8 ± 5.4	0.157	247.3 ± 6.5	0.189
LNE3	**1.5%**	**140.3 ± 3.9**	**0.117**	**148.7 ± 4.2**	**0.145**
LNE4	2%	156.5 ± 4.6	0.123	163.9 ± 5.3	0.138

Values are represented mean± SD, n=3

Table 5.8 Optimization of poloxamer concentration in LNE

Batch	Conc. of poloxamer (w/v)	Initial		After 15 days	
		GS (nm)	PDI	GS (nm)	PDI
TNE5	1%	180.7 ± 6.3	0.159	352.7 ± 6.7	0.254
TNE6	1.5%	113.4 ± 5.2	0.103	194.5 ± 5.4	0.176
TNE7	**2%**	**129.6 ± 4.1**	**0.112**	**136.8 ± 5.2**	**0.130**
TNE8	2.5%	135.9 ± 5.5	0.119	148.4 ± 4.3	0.143

Values are represented mean± SD, n=3

Optimization of Soya lecithin concentration in LNE formulation was done by observing the effect of various concentrations of Soya lecithin (emulsifier) on GS and PDI of formulation. PDI represents uniformity of particle size distribution. Higher value of PDI indicates nonuniform distribution of particle size. PDI less than 0.2 is desirable. PDI values lower than 0.25 indicate a close size distribution providing good stability of nanoemulsions due to the reduced Ostwald ripening (Yilmaz E and Borchert HH, 2005).

As concentration of Soya lecithin was increased up to 1.5%w/v, the GS of formulation was decreased. Further increasing Soya lecithin concentration (2%), it shows increasing GS. LNE3 shows least GS among LNE1, LNE2, LNE3 and LNE4. All these batches were evaluated after 15 days for GS and PDI. Results shows increased value of GS and PDI as compare to initial. After 15 days GS value of batches 1, 2, 3 and 4 were compared, it shows that LNE3 have low GS than others. Thus, LNE3 was taken as optimized batch with 1.5% concentration of Soya lacithin was selected and was taken in final formulation of LNE.

Optimization of poloxamer concentration in LNE formulation was done by observing the effect of various concentrations of poloxamer on GS and PDI of formulation.

Results show that LNE6 have least GS and PDI compare to LNE5, LNE7 and LNE8. These all batches were evaluated for GS and PDI after 15 days also. Results after 15 days indicate

that LNE7 have less GS and PDI value compare to remaining batches. Thus, batch LNE7 containing poloxamer concentration 2% was optimized and thus % of poloxamer in final formulation of LNE was taken 2%.

Thus, final formulation of LNE composed of Capmul MCM (15%), LTG (6.5mg/ml), Soya lecithin (1.5%), Poloxamer (2%) and water (q.s.).

5.3.4 Characterization of Microemulsion and Nanoemulsion

Table 5.9 Characterization of Microemulsion and Nanoemulsion

Evaluation parameters	**TME**	**LME**	**TNE**	**LNE**
Appearance	Clear	Clear	Milky white dispersion	Milky white dispersion
Globule size (nm)	16.69 ± 3.21	14.25 ± 2.73	136.3 ± 4.3	129.6 ± 4.1
PDI	0.089	0.076	0.123	0.112
ZP (mV)	-8.97 ± 0.53	-9.42 ± 0.82	-17.28 ± 0.32	-19.87 ± 0.88
% Assay	98.67± 0.43	99.12 ± 0.52	99.54 ± 0.61	98.83 ± 0.38
pH	6.2 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.5 ± 0.0
Viscosity (cP)	8.23 ± 0.46	7.75 ± 0.32	1.25 ± 0.27	1.43 ± 0.53
% Transmittance	99.09 ± 0.42	99.74 ± 0.53	--	--

Values are represented as mean ± SD, n=3

Figure 5.3 TEM image of (A) TME (B) TNE

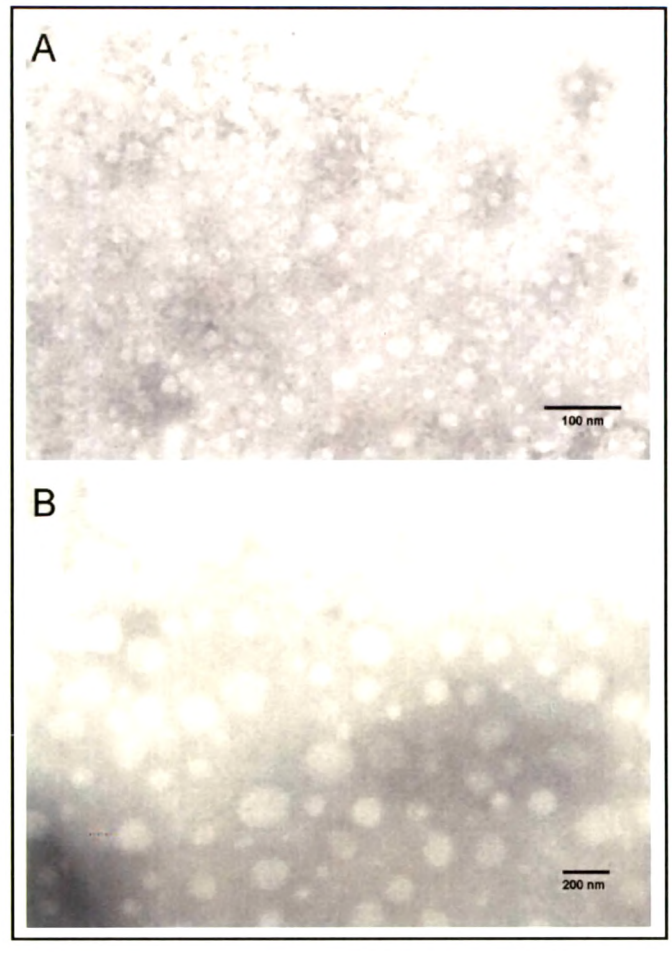


Figure 5.4 TEM image of (A) LME (B) LNE

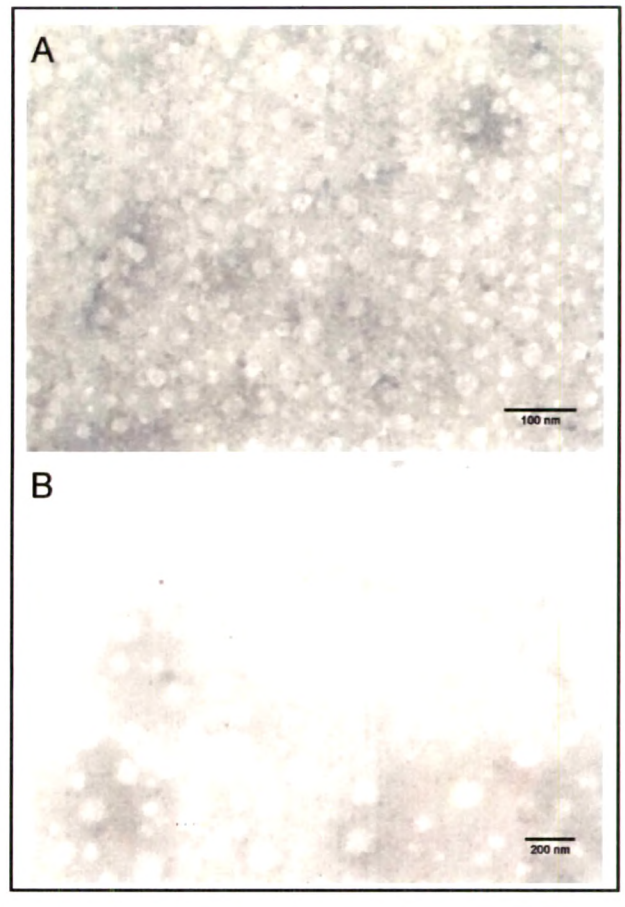


Figure 5.5 *In vitro* release profile of TMD from TS, TME and TNE

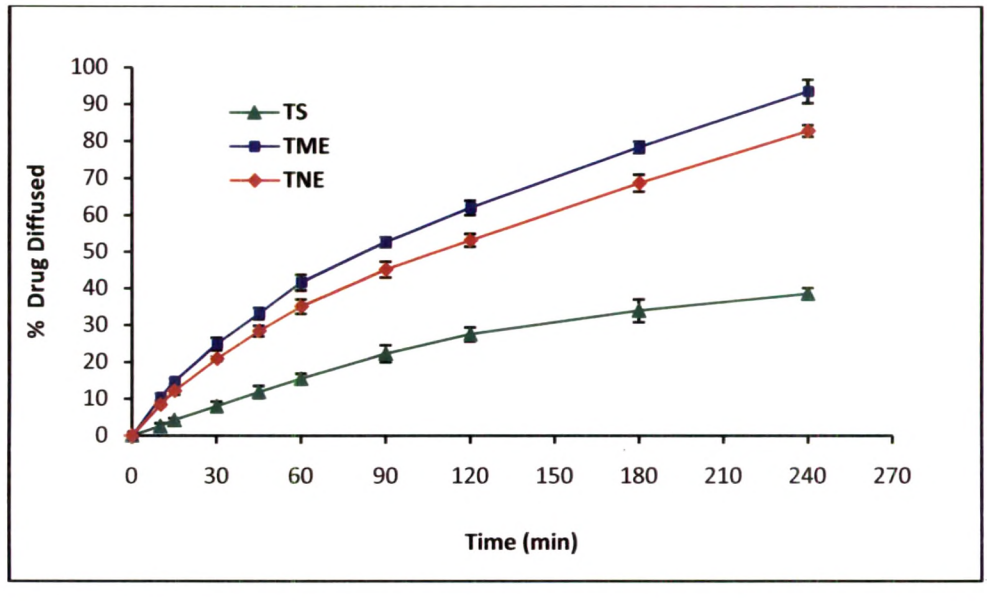


Figure 5.6 *In vitro* release profile of LTG from LS, LME and LNE

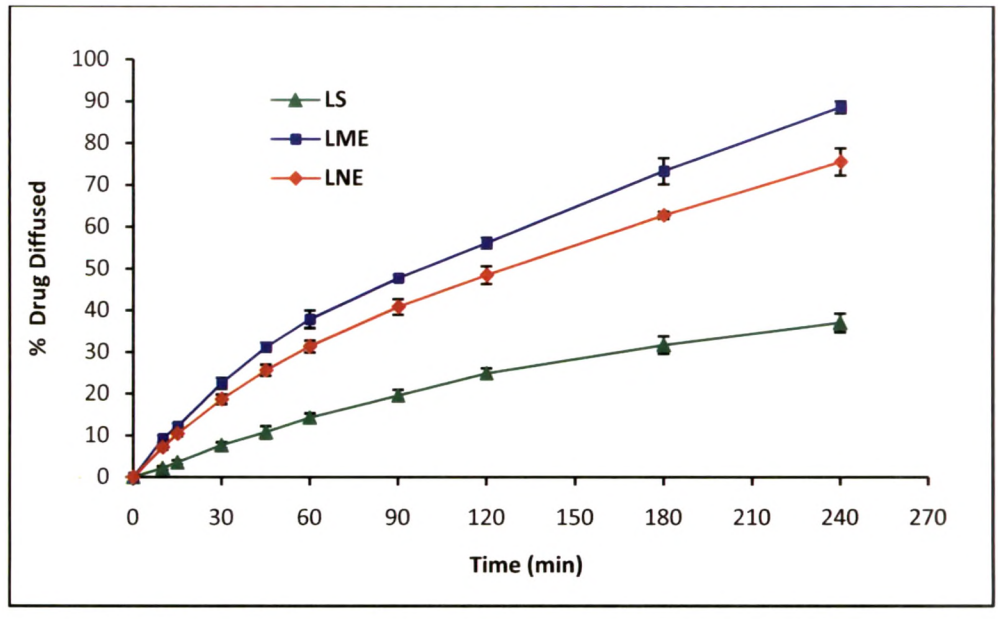


Table 5.10 Mathematical modeling of release profile of TS, TME and TNE

Formulation	Model	R^2 value	Equation
Ts	Zero order	0.949	y = 9.652x + 4.139
	First order	0.972	y = -0.053x + 1.986
	Higuchi	0.92	y = 17.97x
	Hixson Crowell	0.833	y = -0.492x + 2.890
	Korsmeyer peppas	**0.986**	y = 0.865x - 0.854
TME	Zero order	0.965	y = 21.24x + 14.64
	First order	0.957	y = -0.272x + 2.037
	Higuchi	0.966	y = 43.65x
	Hixson Crowell	0.86	y = -0.564x + 2.046
	Korsmeyer peppas	**0.994**	y = 0.686x - 0.414
TNE	Zero order	0.971	y = 18.99x + 11.63
	First order	0.989	y = -0.179x + 1.997
	Higuchi	0.957	y = 37.91x
	Hixson Crowell	0.866	y = -0.556x + 2.209
	Korsmeyer peppas	**0.994**	y = 0.711x - 0.484

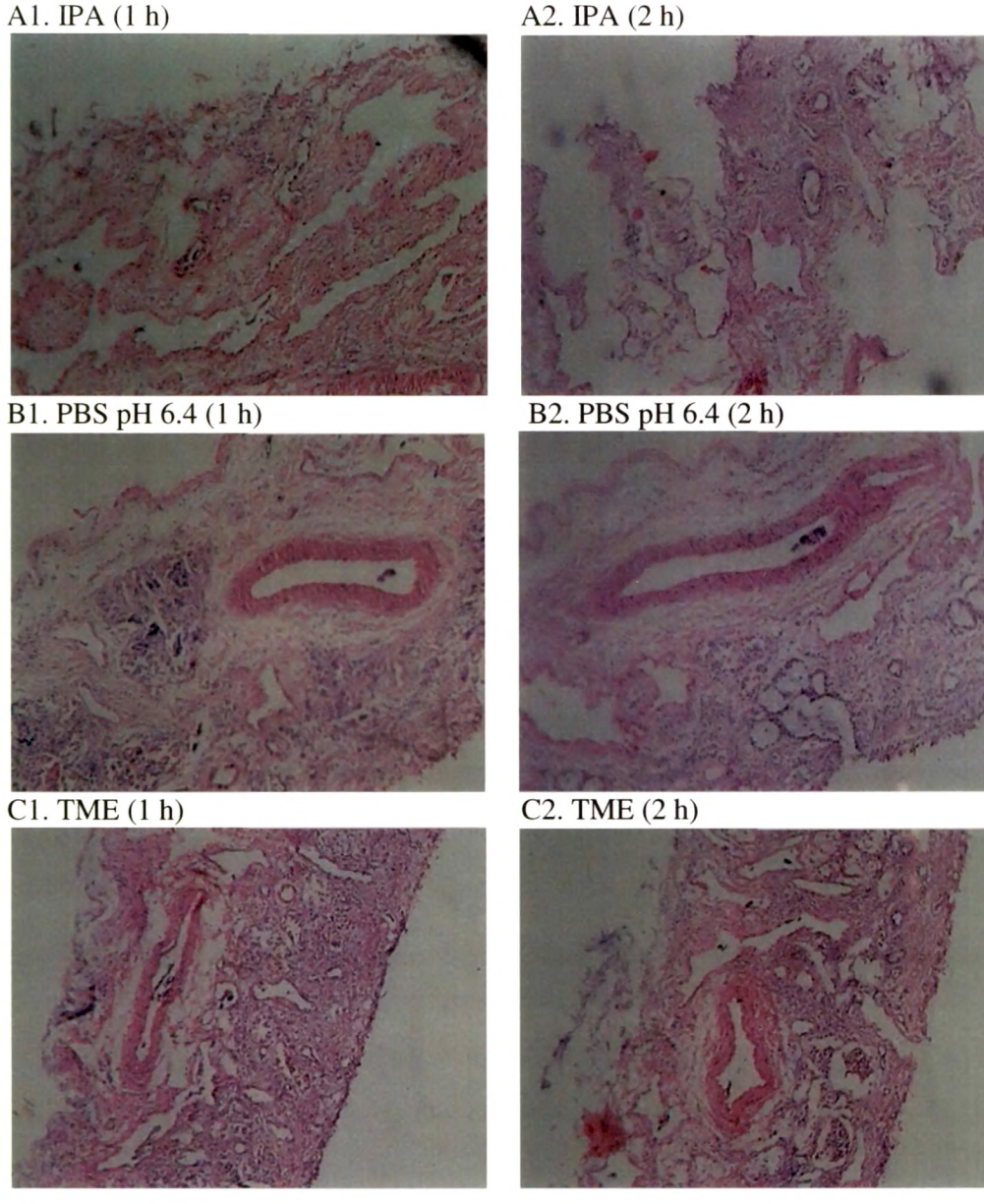
Table 5.11 Mathematical modeling of release profile of LS, LME and LNE

Formulation	Model	R^2 value	Equation
Ls	Zero order	0.966	y = 9.221x + 3.343
	First order	0.984	y = -0.050x + 1.989
	Higuchi	0.913	y = 16.69x
	Hixson Crowell	0.842	y = -0.494x + 2.968
	Korsmeyer peppas	**0.985**	y = 0.898x - 0.897
LME	Zero order	0.971	y = 20.25x + 12.45
	First order	0.973	y = -0.218x + 2.016
	Higuchi	0.957	y = 40.45x
	Hixson Crowell	0.863	y = -0.568x + 2.156
	Korsmeyer peppas	**0.993**	y = 0.713x - 0.456
LNE	Zero order	0.971	y = 17.5x + 10.05
	First order	**0.996**	y = -0.145x + 1.988
	Higuchi	0.952	y = 34.43x
	Hixson Crowell	0.863	y = -0.550x + 2.314
	Korsmeyer peppas	0.994	y = 0.711x - 0.484

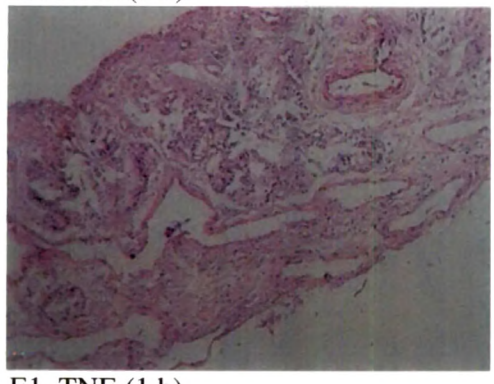
Table 5.12 Flux and diffusion coefficient for Microemulsion and Nanoemulsion

Formulation	Flux	Diffusion coefficient	Ratio DS/Formulation
TS	1.14	0.00023	---
TME	3.27	0.00067	2.86
TNE	3.06	0.00063	2.68
LS	0.84	0.00017	---
LME	2.24	0.00046	2.66
LNE	1.87	0.00038	2.22

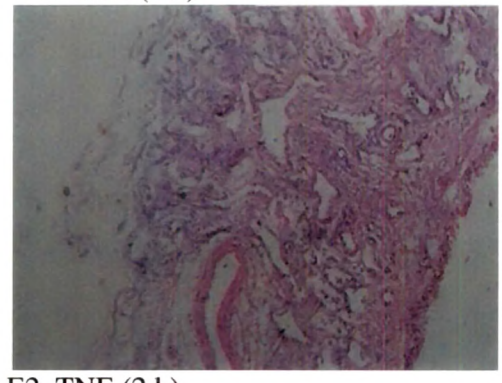
Figure 5.7: Optical microscopy images of sheep nasal mucosa treated with TMD/LTG microemulsion and nanoemulsion formulations



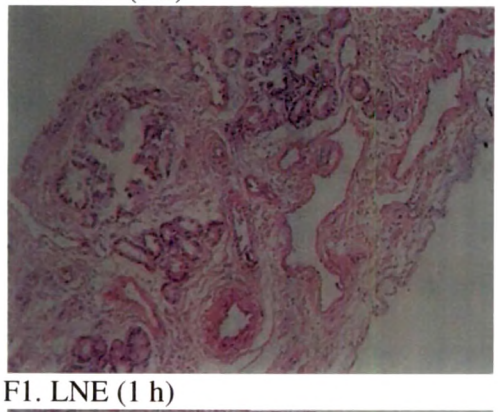
D1. LME (1 h)



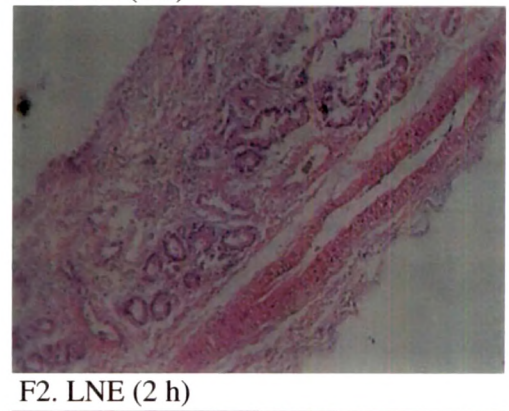
D2. LME (2 h)



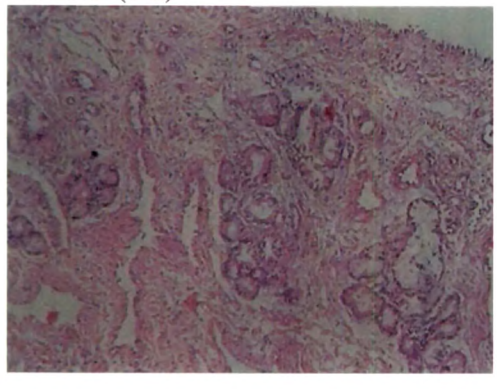
E1. TNE (1 h)



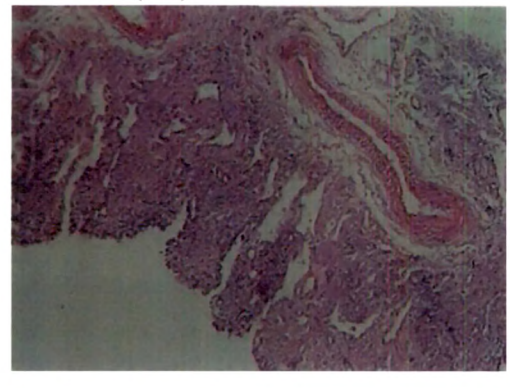
E2. TNE (2 h)



F1. LNE (1 h)



F2. LNE (2 h)



ME and NE of TMD and LTG were characterized for their appearance, globule size, zeta potential, drug content, pH, viscosity, and transmittance, and the results were recorded in Table 5.9. ME formulations have globules in less than 20 nm, while NE formulations have globules in less than 150 nm. Low polydispersity index values suggest narrow size distribution. ZP were lesser than -8.0 mV indicating stability against globule-globule aggregation. (Salim N et al., 2011) The pH of the formulations was found in the range of pH 5 to 6, which is compatible with nasal mucosa. ME demonstrated higher viscosity when compared against NE. NE contains lesser ratio of surfactant/co-surfatcant and hence, have water like consistency. The percentage transmittance of ME was found to be more than 99% and shows that the prepared TME and LME and are isotropic in nature. TEM images (Fig. 5.3 & 5.4) are in agreement with the globule size distribution measured by PCS.

The *in vitro* diffusion study through excised sheep mucosa was performed with an aim to assess the drug release through a biological membrane simulating the actual *in vivo* barrier to drug diffusion. The % cumulative drug diffused across nasal mucosa from TMD and LTG loaded formulations were calculated and shown in Fig .5.5 and 5.6 respectively. The kinetic pattern of the diffusion was studied by fitting % drug diffused in given time in different order kinetics like zero order, first order, higuchi, Hixon Crowell, and Korsmeyer peppas. Regression coefficients of all formulations in different orders were compared and found that the release pattern of TMD and LTG from the formulation across the nasal mucosa followed Korsmeyer peppas order except for LNE which follows first order. This was concluded by higher regression coefficient value in curve fitting. However, apart from Korsmeyer peppas model, zero, first and Higuchi models shows Regression coefficients higher than 0.9 indicating suitability of the said models.

The results show that flux and diffusion coefficients are in the order of ME > NE > DS, which clearly confirms the permeation improvement with ME and NE systems. MEs permit drug loading at saturation solubility and increase their thermodynamic activity favouring partition/permeation into biological membrane. Also, amount of surfactants in ME may lead to tight epithelial junction opening in nasal membrane thereby increasing net flux. However toxicity of ME on nasal epithelial membrane needs to be evaluated. NE demonstrated lower flux than ME however it was more than two times compared to control (DS) indicating suitability for nasal delivery. NE was composed of lecithin as surfactant which is natural body component present in lipid bilayer thus, favouring permeation into biological membrane without affecting normal functioning.

The prepared 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 formulations were shown in Fig. 5.7. The nasal mucosa treated with PBS pH 6.4 showed intact epithelial layer without any damage while mucosa treated with isopropyl alcohol (mucociliary toxic agent) showed complete destruction of epithelial layer and even deeper tissues.

Mucosa treated with MEs (TME and LME) were found to intact with slighter damage of the epithelial layer after 1h. However, after 2 h treatment damage become prominent with loss of epithelial layer. This may be due to high amount of surfactants (30-40%) present in MEs. For Mucosa treated with NEs (TNE and LNE), epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa even after 2 h of treatment as compared with phosphate buffer (pH 6.4) treated mucosa. Thus, the developed NE formulations seem to be safe with respect to nasal administration for repeated administrations. MEs can be use for single use and repeated dosing can affect of integrity nasal epithelium. However further toxicity studies have to be conducted prior to clinical application of the prepared formulations.

5.4 Conclusion

ME and NE of both drugs were successfully prepared by titration and ultrasonication method respectively. MEs of TMD and LTG have very small glouble size (<20nm) and negative zeta potential (< -8mV.) While, NEs have small glouble size (<150nm) and negative zeta potential (< -8mV). The spherical surface of NE and ME was confirmed from TEM. pH of MEs and NEs is compatible with nasal fluid and viscosity of MEs and NEs is suitable nasal administration. *In vitro* release of ME and NE system in nasal mucosal membrane demonstrated prompt and effective release with more than 75 % of drug release in 4 h. Nasal toxicity study revealed safety of NE and NE for repeated and single dosing respectively. However nasal toxicity needs to be evaluated for chronic use. MEs are not suitable for repeated or multiple administrations. The ME and NE were further subjected to stability studies according to ICH guidelines (Chapter 6).

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