



DESIGN DEVELOPMENT AND EVALUATION OF DULOXETINE HYDROCHLORIDE MICROEMULSION FOR INTRANASAL DELIVERY

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ABSTRACT

The objective of this study was to develop novel intranasal microemulsion containing Duloxetine Hydrochloride for the treatment of depression. Duloxetine Hydrochloride microemulsion was prepared by water titration method. Oleic acid was selected as oil phase while tween 80 selected as surfactant and propylene glycol selected as a co-surfactant based on solubility results. Optimized ratio of tween 80: propylene glycol was selected after developing pseudoternary phase diagrams for different ratios and microemulsion were prepared. The prepared microemulsion were evaluated for globule size, zeta potential, viscosity, pH and in-vitro diffusion study. All the parameters showed the suitability of microemulsion of Duloxetine Hydrochloride for intranasal delivery.

KEYWORDS: Duloxetine Hydrochloride, Depression, Intranasal delivery, Microemulsion.

1. INTRODUCTION

As estimated by WHO, depression shall become the second largest illness in terms of morbidity by another decade in the world. Depression is a common, generally chronic, and debilitating psychiatric condition. It is increasingly recognized that depression affects the entire body including painful physical symptoms that may be part of a broader cluster of symptoms that constitute major depressive disorder not merely emotional symptoms (mood and anxiety). The researchers found that intranasal administration was associated with strong improvement in Depression. Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of medication in the remaining tissues. Thus improving efficacy of the drug and reducing side effects. Intranasal drug delivery—practiced for thousands of years, and given a new impact of life. Many scientists have reported evidence of nose-to-brain transport. Many previously abandoned potent CNS drug candidates promise to become successful CNS therapeutic drugs via intranasal delivery. In comparison with oral administration, intranasal drug delivery may provide an improvement in bioavailability and rapid onset of action.^[1] In recent years, systemic drug delivery through nasal route has received a lot of attention, because of its advantages including rapid absorption, avoidance of hepatic first pass metabolism and ability for preferential drug delivery to brain via the olfactory region.^[2,3] Duloxetine Hydrochloride is a selective serotonin and

norepinephrine reuptake inhibitor. Duloxetine was developed for the management of depression.^[4] Duloxetine Hydrochloride aqueous solubility is very poor. Hence solubility enhancement is necessary for intranasal delivery. Microemulsion seems to be a convincing approach for administration of Duloxetine Hydrochloride. Microemulsion is defined as a clear, transparent, thermodynamically stable dispersion of oil, surfactant, co-surfactant and water.^[5] The objective of this investigation was to prepare and optimize intranasal Duloxetine Hydrochloride microemulsion by using various physicochemical parameters including globule size, zeta potential, pH, viscosity, in-vitro drug release study.

2. MATERIALS AND METHOD

2.1 Material

Duloxetine Hydrochloride was obtained from Lupin Pharmaceuticals, Mumbai, India. Tween 80, Tween 20, Span 80, Propylene glycol, Polyethylene glycol were purchased from Loba Chemie pvt. Ltd. Mumbai. Oleic acid purchased from Research lab fine chem Industry Mumbai.

2.2 Determination of solubility of drug^[6]

The solubility of Duloxetine Hydrochloride in various components (oils, surfactants and co-surfactants).

i. In Oils

The solubility of Duloxetine Hydrochloride in each of

the various selected oils Oleic acid, capmul MCM, Labrafac were determined separately by adding an excess amount of drug in 3 ml of Oil in a 10 ml capacity stopper vials and was mixed using a vortex mixer. Further kept for 24 hour at room temperature to reach equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 15 min followed by filtration. The filtrates were diluted with methanol and Duloxetine Hydrochloride solubility was subsequently quantified by UV spectrophotometer.

ii. In surfactants and Co-surfactants

Propylene glycol, Polysorbate 20 (Tween20), Polysorbate 80 (Tween 80), Polyethylene glycol (PEG 400). The solubility of Duloxetine Hydrochloride in each of the various selected oil, surfactants and co-surfactants were determined separately by adding an excess amount of drug in 10 ml surfactants and co-surfactants in a 10 ml capacity stopper vials and was mixed using a vortex mixer. Further kept for 24 hour at room temperature to reach equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 15 min followed by filtration. The filtrates were diluted with the same solvents in which the solubility is performed and Duloxetine Hydrochloride solubility was subsequently quantified by UV spectrophotometer.

iii. Selection of Oils, Surfactants for Formulation Study^[7,8]

Oils and surfactant/co surfactant which shown higher solubility for Duloxetine HCL in the above experiment was selected for further formulation study of microemulsion. These were included, oleic oil selected as a oil, Tween 80 as a surfactant and propylene glycol as a cosurfactant.

iv. Determination of the concentration of oil, surfactant, co-surfactant and water phase for formulation of Duloxetine Hydrochloride microemulsion.

On the basis of solubility studies done above, we have chosen the oil phase, surfactant and cosurfactant needed to form microemulsion Selection was based on maximum solubility in each of the phase. To evaluate the concentration of the excipients viz Oil phase, Surfactants and Cosurfactants, Pseudo Ternary diagram were plotted.

2.3 Construction of Pseudo-Ternary Phase Diagrams^[9]

The pseudo-ternary phase diagrams were constructed using water titration method to determine the Microemulsion region and to detect the possibility of making Microemulsions with different possible compositions of oil,(S/Cos) and water.^[10] The ratios of surfactant to co-surfactants were chosen to be 1:1, 2:1 and 1:2 and such mixtures of Surfactant/cosurfactant were mixed with the oil phase to give the volume ratios of 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. Water was added drop by drop and stirred using a magnetic stirrer until a homogeneous dispersion

was obtained. After each addition, the system was examined for the physical appearance. The end point of the titration was the point where the dispersion becomes cloudy or turbid. The quantity of the aqueous phase required to make the mixture turbid was noted. The percentages of the different incorporated pseudo phases were then calculated and the same procedure was followed for the other Surfactant/co-surfactant ratios. The ternary phase diagrams were constructed by using the Chemix software (Chemix school trial version 3.0).

2.4 Characterization of Microemulsion^[11]

The Microemulsion were evaluated for the following characteristics.

i. Optical Transparency

Optical transparency of the formulation was determined by inspecting the sample in clear and transparent container under the presence of light against reflection into the eyes.

ii. Viscosity Measurement

The Viscosities of Microemulsions were measured using a Brookfield rotational viscometer (LV2, Brookfield Inc., USA). The viscosity of all the microemulsions were measured at 25°C at 12 rpm using small volume adapter with Spindle S- 18 and in triplicate.

iii. Phase Separation

Microemulsion system were subjected to centrifugation (Remi Motor, Mumbai) at 3000 rpm for a period of 2 hours and examined for any evidence of phase separation.

iv. Determination of pH

A 10% dispersion of formulation was prepared in distilled water and pH was determined by using pH meter which was prior standardized with standard buffers of pH. Measurement of.

v. Globule Size

The average globule size of the microemulsions was determined by Zetasizer Nano-ZS (Malvern Instruments, UK). Measurements were carried at an angle of 90° at 25°C. Microemulsion was diluted with double distilled water to ensure that the light scattering intensity was within the instrument's sensitivity range. All the measurement was carried out at 25°C. The polydispersity index of the formulation was determined by the same instrument. The width of the size distribution was indicated by the polydispersity index (P.I).^[13]

vi. Measurement of zeta potential

The zeta potential was determined to verify stability of microemulsion due to charge interaction. Zeta potential was measured by using Zetasizer Nano-ZS (Malvern Instruments, UK). The measurement was performed at 25°C.

vii. In Vitro diffusion study

The use of natural membranes is very important for predicting the potential drug release characteristic. Freshly excised sheep nasal mucosa was obtained from slaughter house and dipped immediately in phosphate buffer (pH 6.8).^[14] Cartilages were removed properly and the mucosal membrane was isolated and washed with phosphate buffer (pH 6.8). *Ex-vivo* drug diffusion study was performed using a Franz-type diffusion cell with a diameter of 10 mm and mucosa thickness of 0.20 mm. The tissue was stabilized in phosphate buffer (pH 6.8). The receptor compartment was filled with 10 ml diffusion media (phosphate buffer pH 6.8). 1ml ME was placed in donor compartment. Samples from the receptor compartment were withdrawn at periodic time intervals, filtered through 0.45 μ m nylon filter paper and analyzed using a UV-Visible spectrophotometer at 289 nm. Each removed sample was replaced by an equal volume of diffusion medium. Amount of Duloxetine Hydrochloride released at various time intervals was calculated with the help of calibration curve with Phosphate buffer and plotted against time.

3. RESULTS AND DISCUSSION

3.1 Solubility of drug

The solubility of Duloxetine Hydrochloride in different oils, surfactants and co-surfactant was determined. The solubility of Duloxetine Hydrochloride was found to be highest in oleic acid, Thus, oleic acid was selected as the oil phase for the development of microemulsion formulation. Among the surfactants studied, Tween 80 showed higher solubility for Duloxetine Hydrochloride and it improve nasal absorption when Tween 80 was used one of the ingredient.^[11] Tween 80 surfactant with HLB 14 was selected as surfactant and depending on the solubility results Propylene Glycol was selected as co-surfactant, it acts as a permeation enhancer.

Table 1: Solubility of Duloxetine Hydrochloride in various excipients.

Material	Solubility (mg/ml)
Labrafac	5.46
Oleic acid	50
Capmul MCM	20
Tween 80	70
Tween 20	30
PG	90
PEG	50

3.2 The pseudoternary phase diagrams

Plotting of Pseudo ternary Phase diagram for formulation of Microemulsion with Smix ratio (1:1, 1:2, 2:1). Pseudo ternary phase diagram shown in following figures.

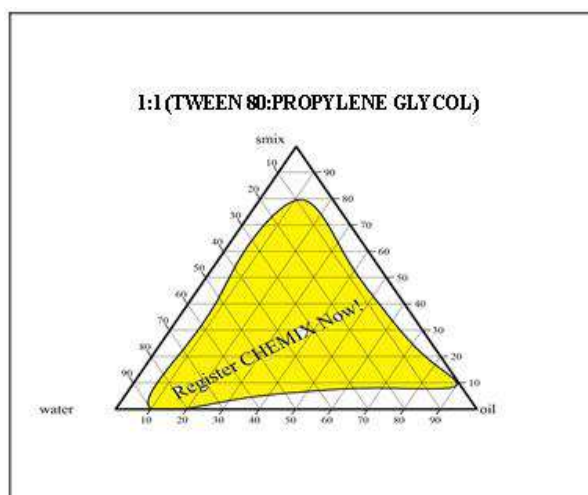


Fig. 1 Pseudoternary phase diagram using oleic acid as oil, Tween 80 as surfactant, propylene glycol as cosurfactant and water (Tween 80: propylene glycol = 1:1).

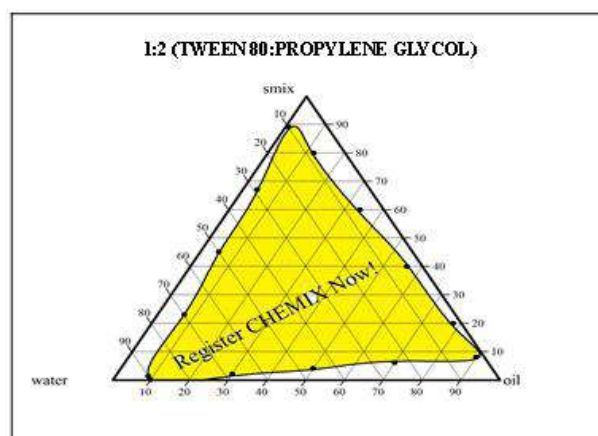


Fig. 2 Pseudoternary phase diagram using oleic acid as oil, Tween 80 as surfactant, propylene glycol as cosurfactant and water (Tween 80: propylene glycol = 1:2).

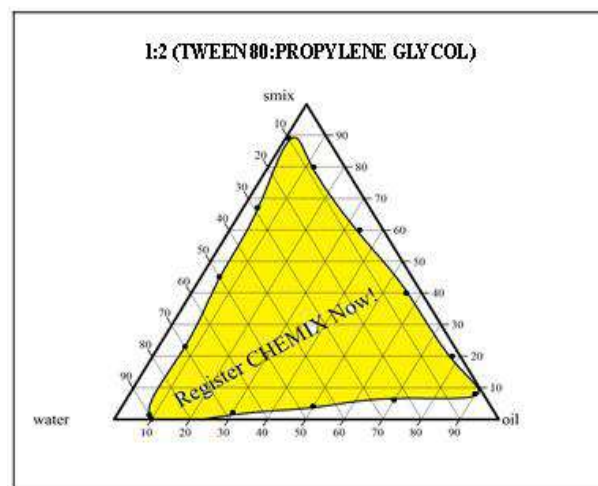


Fig. 3 Pseudoternary phase diagram using oleic acid as oil, Tween 80 as surfactant, propylene glycol as cosurfactant and water (Tween 80: propylene glycol = 2:1).

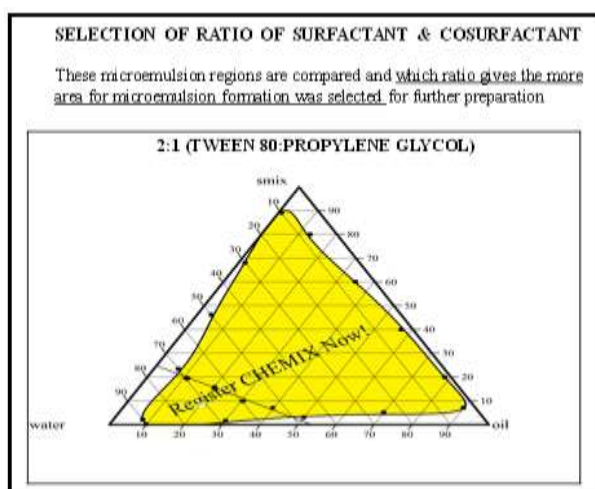


Fig. 4: Pseudoternary phase diagram using oleic acid as oil, Tween 80 as surfactant, propylene glycol as cosurfactant and water (Tween 80: propylene glycol = 2:1, 1: 1 and 1:2).

Table 2: composition of selected ratio of microemulsion formulation.

INGREDIENTS	ME1	ME2	ME3	ME4
DULOXETINE HYDROCHLORIDE	20	20	20	20
OLEIC ACID	8	10	15	20
TWEEN 80	34.66	40	43.33	46.66
PROPYLENE GLYCOL	17.33	20	21.66	23.33
WATER	40	30	20	10

3.3 Characterization of Microemulsion

i. Optical Transparency- Optical transparency of the formulation was determined by inspecting the sample in clear and transparent container under the presence of light against reflection into the eyes. All the formulation were shown clear and transparent.

ii. Phase separation- The formulations were subjected to centrifugations 3000 rpm for a period of 30 min and examined for any change in phase separation. There is no separation found.

iii. pH determination- A 10% dispersion of formulation was prepared in distilled water and pH was determined by using pH meter which was prior standardized with standard buffers of pH. The pH was found to be in range which close to nasal secretions. The pH of the ME 3 was also near to above stated limit which indicated less chances of irritancy on nasal mucosa.

iv. Viscosity measurement- The Viscosities of Microemulsions were measured using a Brookfield rotational viscometer (LV2, Brookfield Inc., USA). The viscosity of all the microemulsions were measured at 25°C at 12 rpm using small volume adapter with Spindle

The pseudo ternary phase diagrams of different Surfactant/co-surfactant (1:1, 2:1, 1:2) ratio gives microemulsion region. In the above diagrams the ratios of 1:1, 1:2 shown the small microemulsion region as compared to the ratio of 2:1 there for 2:1 ratio of tween 80: propylene glycol was selected for the formulation of microemulsion. The components that showed maximum solubility were further optimized using pseudoternary phase diagram. The zone of ME was obtained. ME systems were obtained by mixing oil, surfactant and cosurfactant together and adding appropriate quantity of CBZ and adding precisely distilled water drop by drop to these oily phases with magnetic stirring at ambient temperature.

S- 18 and in triplicate. Higher viscosity is preferred as it increases residence time but permeation rate also decreases with increase in viscosity and hence formulation should have moderate viscosity. Reported values indicates viscosity in between 100 and 200 cps is suitable for nasal administration.^[11]

v. Globule size determination- The average globule size of the microemulsions was determined by Zetasizer Nano-ZS (Malvern Instruments, UK). Result of globule size indicated that smallest globule size was obtained with formulation ME 3 with PDI 0.269, which is close to zero, indicating that the prepared ME had uniform globule size and thus it was selected for further studies as faster permeation is expected when the globule size is small.

vi. Measurement of zeta potential- The zeta potential was determined to verify stability of microemulsion due to charge interaction. Zeta potential was measured by using Zetasizer Nano-ZS (Malvern Instruments, UK). Zeta potential was negative which indicated the stability of formulation as there were less chances of globules aggregation.

Table 3: Physicochemical parameters of ME.

Parameters	ME1	ME2	ME3	ME4
Optical Transparency	Transparent	Transparent	Transparent	Transparent
Phase separation	No phase separation	No phase separation	No phase separation	No phase separation
pH	5.29	6.39	5.92	5.67
Viscosity (cps)	190.2	153.6	192.5	177.3
Globule size (nm)	76.79	82.79	57.49	131.7
zeta potential	-18	-14.1	-16.1	-19.8

3.4 Ex-vivo diffusion through sheep nasal mucosa

Ex-vivo diffusion study was performed by using sheep nasal mucosa for optimized formulation. A biphasic release profile was obtained in which initial faster release was due to solubilized drug in continuous phase while

slower rate was due to Duloxetine Hydrochloride release from the oil droplets. Duloxetine Hydrochloride from ME permeates rapidly (more than 80% of drug in 50 min) through nasal mucosa in comparison CBZ solution (less than 40% in 90 min).

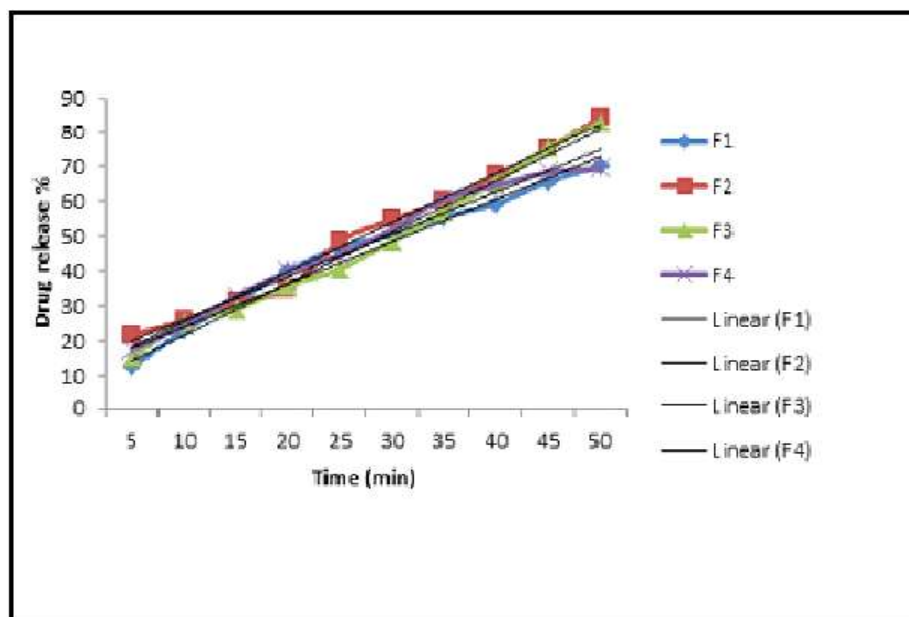


Fig. 5: *In Vitro* drug release.

CONCLUSION

Use of the intranasal route is being exploited extensively to target drugs to the brain for the treatment of various CNS related diseases. Exploration of the olfactory region of nasal mucosa is adding fuel to this research. Duloxetine Hydrochloride microemulsion formulations were successfully developed by the spontaneous emulsification technique. The results of diffusion studies confirmed rapid drug release of Duloxetine Hydrochloride through nasal mucosa, thus providing a rapid antidepressant effect. Intranasal delivery may be considered as an effective route to deliver drugs rapidly and efficiently into the brain for the treatment of depression without accessing through the BBB.

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