Chapter 6 (Part- A)

Characterization of oral SMEEDS

Management of Dyslexia and ADHD

6.1 Introduction

The optimized SMEDDS was further characterized for the different parameters to ascertain its stability and to predict its*invivo* performance.

6.2 Characterization of Oral SMEDDS

6.2.1Thermodynamic Stability Testing

To evaluate thermodynamic stability, Modafinil loaded SMEDDS was subjected to heating cooling cycle, centrifugation test and freeze thaw cycle. (1-3)Moreover, physical stability of SMEDDS was continuously monitored throughout experiment. Various parameters like phase separation, turbidity/haziness, globule size, zeta potential etc. were observed.

6.2.1.1 Heating Cooling Cycle

Modafinil loaded SMEDDS formulations of optimized batch were subjected to the three cycles at refrigerator temperature (4˚C) and at stability chamber (45˚C)for not less than 48 hrs. These formulations were observed for its stability.

6.2.1.2 Centrifugation Test

Optimized SMEDDS was centrifuged at 3500 rpm for 30mins and was analyzed for drug precipitation and/or phase separation.

6.2.1.3 Freeze Thaw Cycle Stress Test

The optimized formulations were subjected to freeze thaw cycle stress test by exposing to a temperature from -20˚C and +25˚C with storage for not less than 48hrs.Stability of these formulations were finally observed.

6.2.2 Self-emulsification Time

Optimized formulations were further subjected to dispersibility test i.e. efficiency of self-emulsification. The formulations were observed visually for any phase separation or color change.The time required to form complete dispersion in water or dissolution media was recorded and considered as self-emulsifying time.The self-emulsification efficiency of SMEDDS was further analyzed by using an USP type II dissolution apparatus.(3) The

specified amount of the SMEDDS (5ml) was added to 250 ml of water at 37±0.5˚Cat50 rpm. After being equilibrated, the competence of self-emulsification and appearance along with dispersibility was observed visually as per the grading systems shown in Table 6.1.

Table 6.1 Classification of SMEDDS based on Dispersibility

Grade	Dispersibility and Appearance
A	Clear, transparent in appearance and rapid forming
B	Bluish white appearance, less clear emulsion and rapid forming
C	Fine milky emulsion forms within 2 min
D	Grayish white emulsion or bright white emulsion with a slight oily appearance
E	Poor emulsification with or without large oils droplets on the surface

6.2.3 Robustness to Dilution

The SMEDDS of Modafinil was subjected to dilution (100 times) in various media like 0.1N HCL, distilled water and pH 7.4 phosphate buffer.(3, 4)The diluted SMEDDS were stored at room temperature for 24 hours and analyzed for any sign of drug precipitation or phase separation, then ordered as clear (with or without bluish tinge and transparent), non-clear (turbid), stable (no precipitation after 24 hours), or unstable (precipitate in 12 hours). Globule size and PDI was checked for diluted SMEDDS and is represent in Table 6.3.

6.2.4 Dye Solubility Study

Dye solubility study is also known as the staining test in which SMEDDS was diluted with aqueous phase (1:100 times) and sprinkle/add the dye into the surface of the dispersion to study the nature of the continuous phase. Test was performed by addition of 2-3 drops of water soluble dye (methyl orange) to the optimized formulation and after 5 minute visual observation was done.(4)

6.2.5 Globule Size

SMEDDS was diluted 100 times with aqueous phase and the globule size and polydispersity Index (PDI) were determined by photon correlation spectroscopy (Malvern Zeta Sizer Nano ZS 90, Malvern Instruments, Malvern, UK). All measurements were performed in triplicate. The results are presented as mean size ± SD. (3, 4)

6.2.6 Zeta Potential

The Zeta potential (ZP) is a measure of the electric charge at the surface of the particles indicating the physical stability of colloidal systems. ZP was measured by using a Zeta Sizer Nano ZS90 (Malvern Instruments, Malvern, UK).(2, 4)The working principle is based on electrophoretic mobility (μm/s) of the globules, which was converted to the zeta potential by in-built software based on Helmholtz-Smoluchowski equation. Each SMEDDS sample was diluted 100 times with double distilled water and was placed in disposable zeta cell. All the measurements were performed in triplicate and the results are expressed as mean ± SD.

6.2.7 pH Measurement

The pH of 100 times diluted (with double distilled water) SMEDDS was measured using a pH meter (Electro lab). All the measurements were performed in triplicate and the results are expressed as mean ± SD.

6.2.8 Percentage Transmittance

SMEDDS were diluted (10, 50, 100 times) with distilled water and % transmittance at 650nm was measured against double distilled water as blank by UV spectrophotometer (UV 1700, Shimadzu, Japan). (1) All the measurements were performed in triplicate and the results were expressed as mean ± SD.

6.2.9 Conductance

For the conductivity measurements, SMEDDS was diluted with a 0.01N aqueous solution of NaCl. The electrical conductivity (σ) was measured by using an electro-conductometer at 25ºC temperature(CM 180 conductivity meter, Elico, Mumbai, India). (3, 5) All the measurements were performed in triplicate and the results were expressed as mean ± SD.

6.2.10 Viscosity

Brookfield viscometer, DV-1 was used to evaluate viscosity of the Modafinil SMEDDS at 25°C temperature. Before and after dilution (10 and 100 times dilution) of formulation with aqueous phase, viscosity was measured with spindle 64 at rotation speed of 100 rpm.(5, 6)

6.2.11 Cloud point

Cloud point temperature (Tc) was determined by diluting 1mL of SMEDDS to 100mL with distil water and was placed in a water bath with the gradually increasing the temperature. Cloud point was measured as the temperature at which there was a sudden appearance of cloudiness visually. After the temperature exceed beyond the cloud point, the sample was cooled, and then was heated again to check the reproducibility of the measurements. (7-9)All the measurements were performed in triplicate and the results were expressed as mean ± SD.

6.2.12 Assay

Optimized SMEDDS was analyzed to determine the content of Modafinil in the formulation. Accurately weighed SMEDDS were diluted in the methanol and drug content was determined by validated HPLC method. All the measurements were performed in triplicate and the results were expressed as mean ± SD. (3)

6.2.13 Transmission Electron Microscopy (TEM)

The morphology of SMEDDS was investigated using TEM (Jeol, JEM - 1011). Briefly, it was carried out by operating at acceleration voltage of 100kv. The formulation was diluted up to 50 times with distilled water for the analysis. Approximately 2 min after sample deposition (1-2 µl), the carbon-coated copper grid (300 mesh, 3mm) was tapped with filter paper to remove surface water and air-dried. The image was taken by Transmission Electron Microscope.(2, 10)

6.3 Result and Discussion

6.3.1 Thermodynamic Stability Testing

Results of thermodynamic stability studies, robustness to dilution and assessment of efficiency of self-emulsification are summarized in Table 6.2. It was concluded that, SMEDDS were stable after the heating cooling cycle and therefore, further analyzed for the centrifugation test and freeze thaw study. SMEDDS did not show any phase separation after centrifugation and freeze thaw stress test. Test concluded that the prepared formulations showed good stability.

Table 6.2 Thermodynamic Stability Testing for SMEDDS

Sr No.	Test	Observation	Inference
1	Heating Cooling Cycle	Clear without any sign of turbidity	Stable
2	Centrifugation Test	No phase separation	Stable
3	Freeze Thaw Stress Testing	No precipitation or color change	Stable

6.3.2 Self-emulsification Time and Dispersibility Grade Assessment

The formulation passed the dispersibility test with grade A, optically clear microemulsion when the study was carried out in water for the optimized SMEDDS formulation. Moreover, the optimized SMEDDS formulation was easily self-emulsified in the distilled water (37±0.5°C) with self-emulsification time of 24 ± 1.5 sec (<1 min).As reported in various literatures, when dispersed globules travelled through the gastrointestinal tract, it should disperse completely and quickly to form a fine microemulsion with the aid of GI fluid under mild agitation of GIT due to peristaltic activity.

6.3.3 Robustness to Dilution

As shown in Table 6.3, optimized SMEDDS formulation of Modafinil was found to be robust to dilution (dilution factor 100) in water, 0.1N HCL and pH 7.4 phosphate buffer and doesn't show any sign of precipitation/turbidity and phase separation and remained clear and stable at least for 24 hours.

Table 6.3 Effect of Dilution Medium (Dilution Factor 100) on SMEDDS

Sr. No	Solvent	% Transmittance	Globule Size (nm)	PDI
1	Distilled water	98.97 ± 0.28	18.97±0.47	0.186 ± 0.21
2	0.1 N HCL	98.82 ± 0.19	18.69 ± 0.46	0.119 ± 0.06
3	Phosphate Buffer pH 7.4	98.73 ± 0.37	18.59 ± 0.58	0.221 ± 0.22

6.3.4 Dye Solubility Study

On the addition of the water-soluble dye methyl orange, it was observed that dye distributed uniformly throughout the diluted SMEDDS without any clumps. Rapid incorporation of a water soluble dye into the O/W type system of SMEEDS whereas with W/O type, the dye forms microscopically visible clumps. The reverse happens on addition of an oil-soluble dye. These tests essentially identify the continuous phase. Result suggest that the continuous phase was water. Thus, it can be concluded that microemulsion formed after dilution of SMEEDS was O/W type.

6.3.5 Globule Size and Zeta Potential Determination

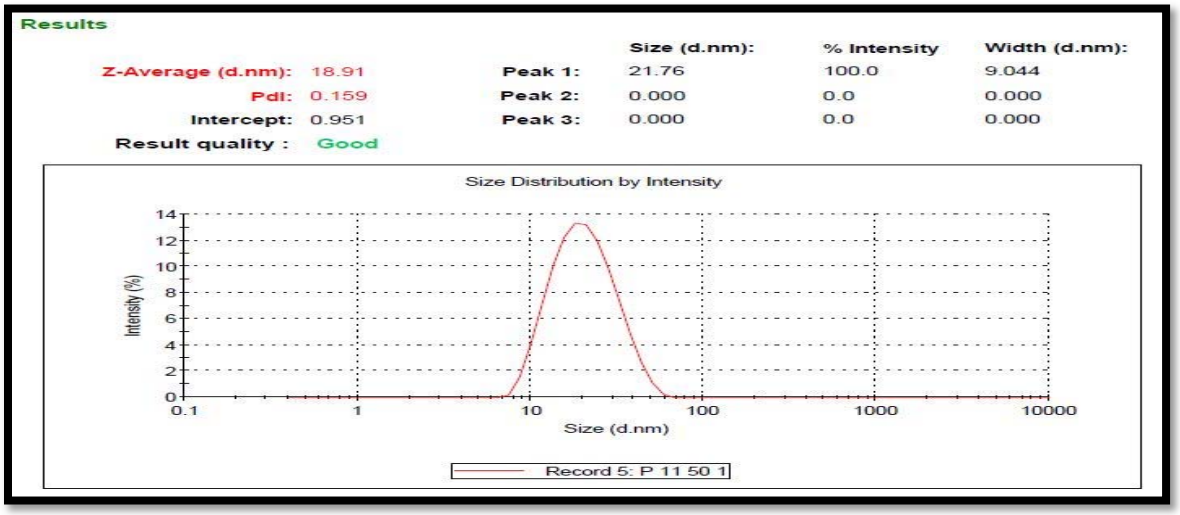

Fig. 6.1 Globule Size for 100 Times Diluted SMEDDS

The Z-average size of the resultant diluted SMEDDS (100 times) was 19.21±0.47 nm with PDI value of 0.186 ± 0.27, globule size of one of the three sample was shown in Fig.6.1, indicate that the system has narrow size distribution with very good uniformity of the globule size. As reported in literatures, small particle/ globule size of the formulation

enhances the permeation through the mucus membrane and provides larger surface area for drug absorption.

Zeta potential of diluted SMEDDS (100 times) formulation was found to be -2.73±0.14 mV, zeta potential of one of the three sample was shown in Fig.6.2. In conventional SMEDDS, the charge of the oil droplets is negative due to the presence of free fatty acids.

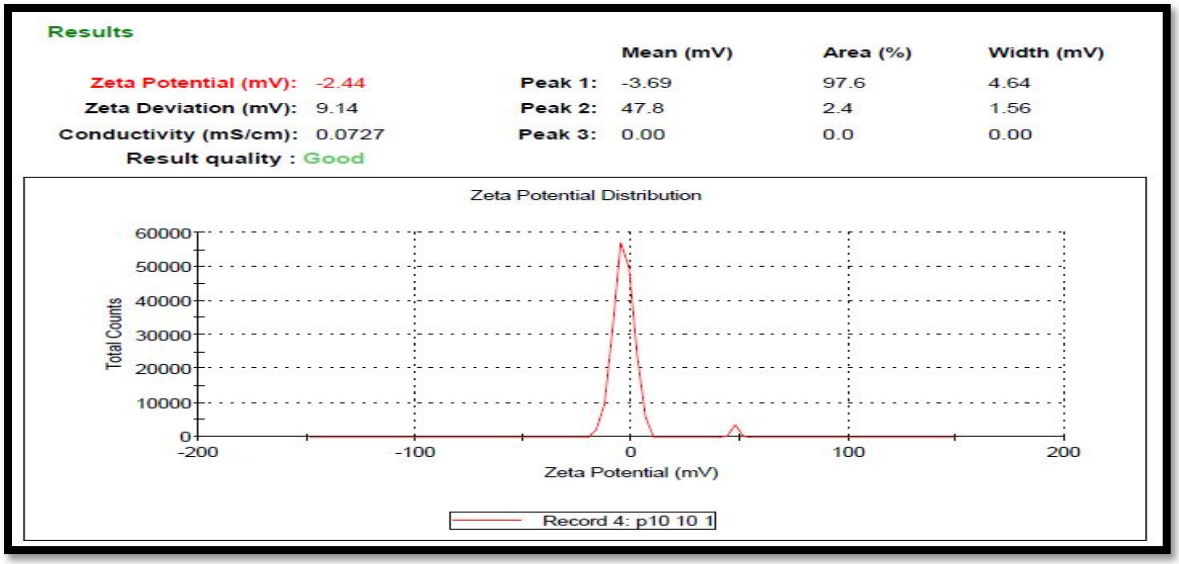

Fig. 6.2 Zeta Potential for 100 Times Diluted SMEDDS

6.3.6 pH measurement

The excipients used in the formulation decide the pH of the final preparation. Change in pH may change zeta potential of formulation that may affect the stability of preparation. Therefore,pH is also responsible for stability of SMEDDS. Optimized SMEDDS formulations showed consistent pH values pH6.28± 0.13. Because it was nearer to neutral pH, stability of SMEDDS may not be affected by pH.

6.3.7 Percentage Transmittance

The value of % transmittanceof 10, 50 and 100 times diluted SMEDDS is represent in Table 6.4 & was found to be nearer to 100%, indicated that the system was optically clear.

Table 6.4 Percentage Transmittance of Diluted SMEDDS with Distilled Water

Sr. No.	Dilution Factor	% Transmittance
1	10 times	99.11 ± 0.19
2	50 times	99.53 ± 0.14
3	100 times	99.67 ± 0.17

6.3.8 Conductance

Conductance measurement of diluted SMEDDS assess whether the formulated self-emulsifying microemulsion having oil-continuous or water continuous phase. If the water phase is continuous then it shows maximum conductance where in case of oil, continuous phase the conductance is minimum. Conductivity value of optimized SMEDDS formulation was found to be 56.21± 0.37 µsec/cm. Optimized SMEDDS formulation was water continuous i.e. o/w system.

6.3.9 Viscosity

As shown in Table 6.5, viscosity of the formulation gradually decreased with an increase in the aqueous phase.The structure and type of microemulsion system can be characterized by rheological measurements as a function of the aqueous phase. This study revealed that on *in vivo* administration of SMEDDS, it may dilute with the stomach fluid, results in decrease in viscosity and thereby absorption may be faster. Moreover, viscosity of SMEDDS system depends on the type of the surfactants that were used.

Table 6.5 Viscosity of the SMEDDS before and after Dilution with Distilled Water

Sr. No.	Content	Viscosity (cP)
1	Initial SMEDDS	1229.3 ± 0.57 cP
2	After 10 times dilution	19.3 ± 0.14 cP
3	After 100 times dilution	0.9 ± 0.09 cP

6.3.10 Cloud Point

The cloud point is the temperature above which the formulation clarity turns into cloudiness andis acrucial factor in the SMEDDS, and responsible for the stable formation of microemulsion*in vivo*. (22)An irreversible phase separation will occur when the temperature is higher than the cloud point, due to dehydration of the polyethylene oxide, leads to poor absorption of drug from GI tract. Preferable cloud point should be above

37◦C, which will avoid the phase separation in the gastrointestinal tract. Cloud point,76.0 ± 3◦C was found for the optimized SMEDDS formulation. Therefore, it may form a stable microemulsion after*invivo* administration of the SMEDDS.

6.3.11 Assay

Assay of the SMEDDS gives the amount of drug present in the formulation. Assay of the prepared modafinil SMEDDS was found to be 99.69 ± 0.23%.

6.3.12 TEM

Morphology of diluted SMEDDS was studied using TEM; image is shown in figure 6.3.

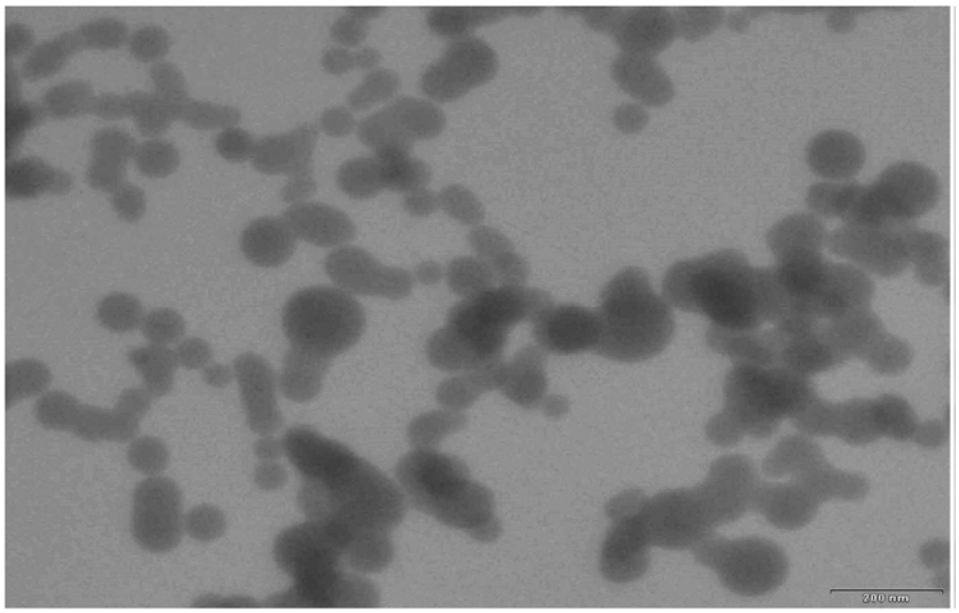

Fig. 6.3 TEM Image of the Diluted SMEDDS of Modafinil

The diluted SMEEDS seems to have uniformly distributed and having spherical shaped globules. Furthermore, no aggregation of the globules indicates the stability of the system. The globule size seemed to be in agreement with the result obtained from globule size analysis using Zetasizer.

Chapter 6 (Part- B)

Characterization of NTB Microemulsion

Management of Dyslexia and ADHD

6.4 Introduction

From the optimization of the formulation, final batch of ME and MME were selected and characterization was done for different parameters to ascertain its stability and predict its *in vivo* performance.

6.5 Characterization of ME and MME

6.5.1 Thermodynamic Stability Testing:

To evaluate the thermodynamic stability, Vinpocetine loaded ME and MME were subjected to heating cooling cycle, centrifugation test and freeze thaw cycle.(11, 12) Test performed same as describe in section 6.2.1.

6.5.2 Robustness to Dilution

Robustness of Vinpocetine loaded ME and MME were evaluated by dilution of specified quantity of formulation with dilution medium (water and sodium acetate buffer pH 5.0) at 100 times. (12) The diluted MEs were stored for 24 hours and observed for any signs of phase separation or drug precipitation, and categorized as discussed in section 6.2.3.Globule size and PDI was also checked for diluted ME and MME and is represent in Table 6.7.

6.5.3 Dye Solubility Study

ME and MME were diluted with aqueous phase (1:100 times) and sprinkled the dye onto the surface of the microemulsion to study the nature of the continuous phase. Test was performed as discussed in section 6.2.4.

6.5.4 Globule Size Measurement

ME and MME were diluted 10 times with aqueous phase and globule size and polydispersity index (PDI) of the dispersion were determined using clear disposable zeta cell by Malvern Zeta Sizer Nano ZS 90 (Malvern Instruments, Malvern, UK). (13-15)All measurements were performed in triplicate recorded. The results are expressed as mean size ± SD.

6.5.5 Zeta Potential Measurement

ZP was measured using a Zeta Sizer Nano ZS 90 (Malvern Instruments, Malvern, UK).Each sample was 10 times diluted with double distilled water and was placed in a disposable zeta cell. (13, 14) All the measurements were performed in triplicate and the results are expressed as mean ± SD.

6.5.6 pH Measurement

The pH of diluted ME and MME (10 times with double distilled water)were measured using pH meter (Electro Lab).All the measurements were performed in triplicate and the results are expressed as mean ± SD.

6.5.7 Percentage Transmittance

Both ME and MME formulation were diluted (10, 50, 100 times) with sodium acetate buffer pH 5.0 and % transmittance at 650 nm was measured against double distilled water as blank by UV spectrophotometer (UV 1700, Shimadzu, Japan).(13)All the measurements were performed in triplicate and the results were expressed as mean ± SD.

6.5.8 Conductance

The electrical conductivity (σ) of the ME was measured using an electro-conductometer at 25ºC temperature (CM 180 conductivity meter, Elico, Mumbai, India). (16-18) All the measurements were performed in triplicate and the results were expressed as mean ± SD.

6.5.9 Viscosity

The viscosity of the ME and MME before and after dilution (10 and 100 times dilution) was measured by using Brookfield viscometer. (13, 16)Method was same as discussed in section 6.2.10.

6.5.10 Cloud Point

Vinpocetine loaded both ME and MME were diluted with water in the ratio of 1:100, and Cloud pointwas measure as the method described in section 6.2.11.

6.5.11 Assay

Optimized formulation of ME and MME systems were analyzed to determine the content of Vinpocetine in the formulation. In brief, 500 µl of ME and MME diluted in the methanol up to 2 ml, further 1 ml of this solution was diluted 100 times with methanol and as per analytical method (discussed in chapter 4) amount of drug was determined by UV spectroscope. Test was performed in triplicate.

6.5.12 Histopathology Study

The nasal-cavity mucosa of a sheep was obtained from the local slaughter house. Within 15 min of the sacrifice of the animal, the nasal cavity was fully exposed by a longitudinal incision through the lateral wall of the nose while avoiding the damage of the septum. Following, the mucosa was carefully removed and immediately immersed in 900 ml of ice-cold Ringer's solution for 15 to 30 min. Sheep nasal mucosa in six pieces with uniform thickness mounted on the Franz diffusion cell. One mucosa was treated with 0.5 ml phosphate buffer saline (PBS) 6.4 and one of the mucosa was treated with isopropyl alcohol and remaining with microemulsion and mucoadhesive microemulsion for 1 hr. After 1 hr the mucosa was 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 taken as positive and negative control respectively. The prepared pathological slides were study under optical microscope for any sign of toxicity of the cells and finally it was evaluated by comparing the results of drug loaded products with positive and negative group.(16)

6.5.13 Transmission Electron Microscopy (TEM)

The morphology of MME was investigated using TEM (Jeol, JEM - 1011) as per the method discussed in section 6.2.13**.** (16, 19)

6.6 Result and Discussion

6.6.1 Thermodynamic Stability Testing

Physical and thermodynamic stability of ME and MME was required to study its performance and to check whether its stability affected by precipitation of the drug or not. If formulation has poor stability, it can affect the formulation performance and leads to phase separation. Hence thermodynamic stability studies were performed by exploring heating cooling cycle, centrifugation test and freeze thaw cycle. Results of thermodynamic stability studies and robustness to dilution are summarized in Table 6.6 and Table 6.7 respectively. It was concluded that, ME and MME formulations were stable after the heating cooling cycle and therefore, further analyzed for the centrifugation test and freeze thaw study. ME and MME formulations do not show any phase separation after centrifugation and freeze thaw stress test. Test concluded that the prepared formulations have good stability without any precipitation of the drug particles, as well as no phase separation and cracking/creaming.

Table 6.6 Thermodynamic Stability Testing for Microemulsion Systems

Sr. No.	Test	Observation		Inference
		ME	MME	
1	Heating Cooling Cycle	Clear without any sign of turbidity	Clear without any sign of turbidity	Stable
2	Centrifugation Test	No phase separation	No phase separation	Stable
3	Freeze Thaw Stress Testing	No precipitation or color change	No precipitation or color change	Stable

6.6.2 Robustness to Dilution

ME and MME formulations of Vinpocetine found to be robust to dilution and doesn't show any sign of precipitation/turbidity and phase separation and remained clear and stable at least for 24 hours. Robustness todilution test is based on the fact that the emulsion is only miscible with the liquid that forms continuous phase. The system is diluted with either the oil or the aqueous phase, whichever is used as the external phase 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. Here, system diluted with aqueous phase and remained clear, represent that the system was O/W type.

Table 6.7 Effect of Dilution Medium (Dilution Factor 100) on Microemulsion Systems

Sr. No.	Solvent	ME			MME		
		% T	Globule Size (nm)	PDI	% T	Globule Size (nm)	PDI
1	Distilled water	99.22 ± 0.20	24.91 ± 0.19	0.152 ± 0.07	97.73 ± 0.42	29.11 ± 0.32	0.163 ± 0.24
2	Sodium Acetate pH 5.0	99.06 ± 0.45	25.53 ± 0.52	0.144 ± 0.09	98.89 ± 0.03	35.81 ± 0.64	0.219 ± 0.81

6.6.3 Dye Solubility Study

On the addition of the water-soluble dye methyl orange, it was observed that it distributed uniformly throughout the diluted ME and MME (O/W microemulsion) and it doesn't produce any clumps, which suggests that the dye distributed uniformly throughout the continuous phase. Thus, it can be concluded that microemulsion was O/W type.

6.6.4 Globule Size and Zeta Potential Determination

Small globule size of the formulation enhances the permeation through the mucus membrane and provides larger surface area by the same for drug absorption. The Z-average size of the resultant 10 times diluted (with distilled water) ME and MME were 19.01 ± 1.11nm and 21.11 ± 1.56 nm with PDI value of 0.287 ± 0.034 and 0.133 ± 0.016 respectively, (one of the result show Fig.6.4 and 6.5), indicating that the system had narrow size distribution and uniform particle size.

The charge of the microemulsion is negative due to the presence of free fatty acids while for MME it was positive due to presence of chitosan. Zeta potential of the 10 times diluted ME and MME systems were found to be -20.7 ± 0.44 mV (one of the result shows in Fig.6.7) and 8.46± 0.49 mV(one of the result shows in Fig. 6.6) respectively. Zeta potential indicates the degree of repulsion between adjacent and similarly charged particles in dispersion.

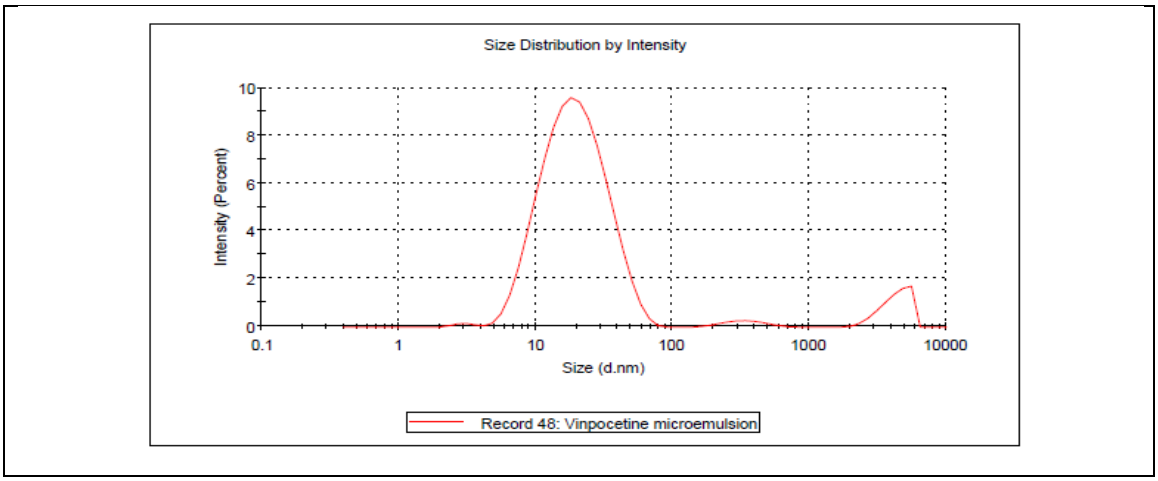

Fig 6.4 Globule size of Vinpocetine loaded Microemulsion (ME)

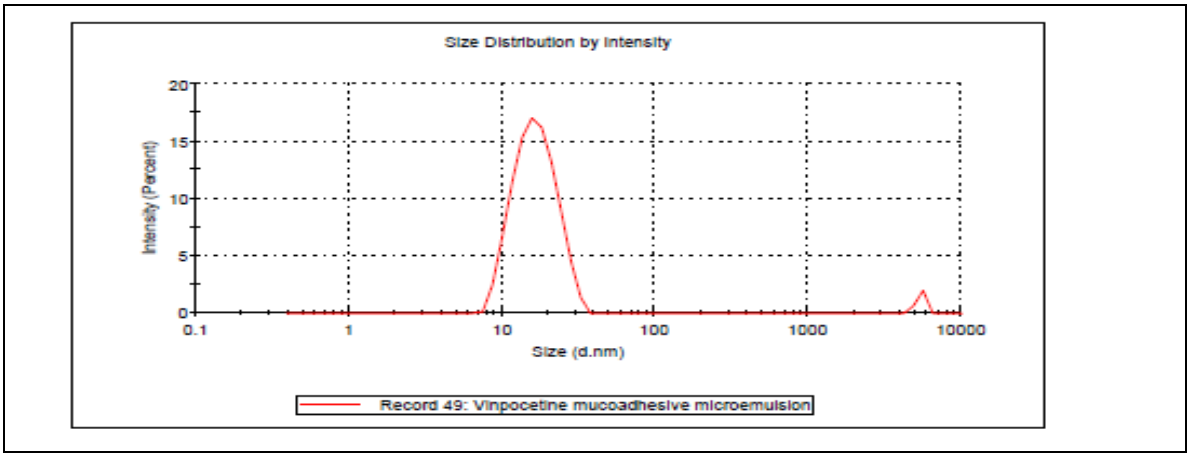

Fig 6.5 Globule size of Vinpocetine loaded Mucoadhesive Microemulsion (MME)

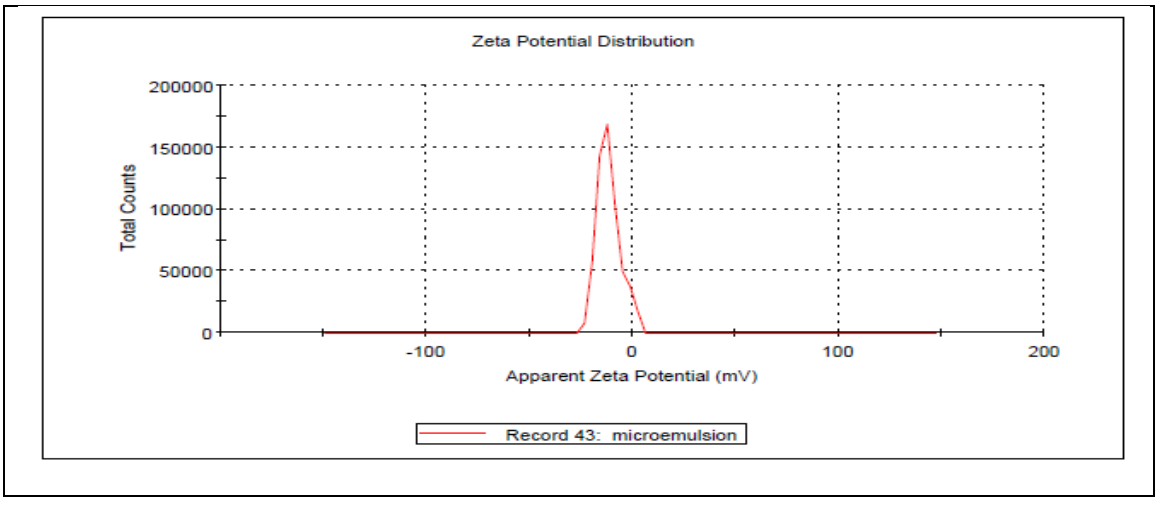

Fig 6.6 Zeta Potential of Vinpocetine loaded Microemulsion (ME)

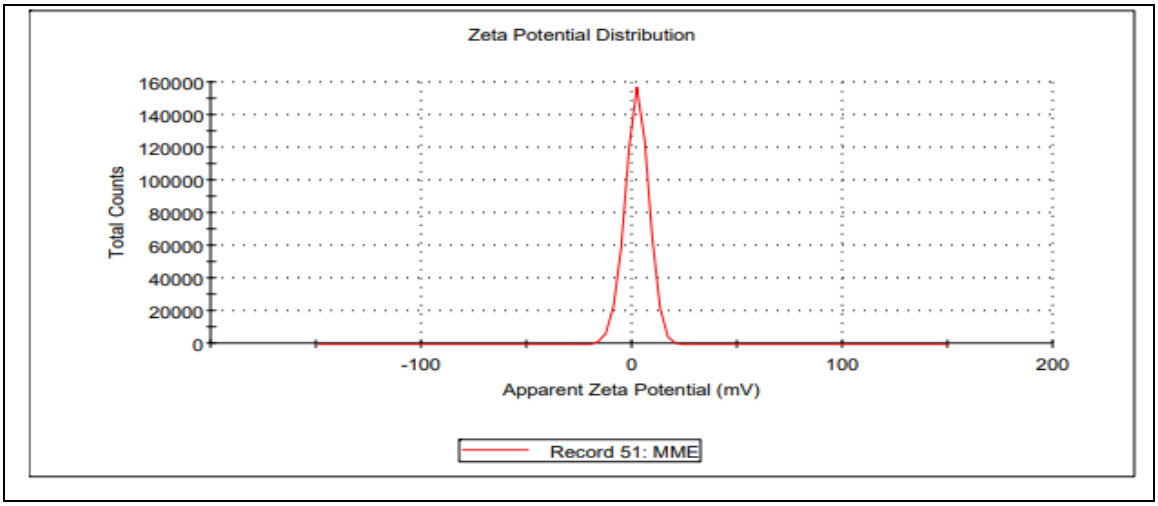

Fig 6.7 Zeta Potential of Vinpocetine loaded Mucoadhesive Microemulsion (MME)

6.6.5 pH Measurement

The excipients used in the formulation decide the pH of the final preparation. Change in pH may change zeta potential of formulation, which may affect the stability of preparation. Therefore, pH is also responsible for stability of microemulsion. Optimized ME and MME formulation shows pH 5.51±0.07 and 5.64±0.09. The pH of the formulations was found to be within the range of nasal cavity secretions and hence would not cause nasal irritation on application. Here pH is an important parameter for nasal administration. Formulations with neutral pH may enhance chances of microbial infection of nasal cavity because at less acidic pH, lysozymes of nasal cavity became inactive. Therefore, pH adjustment was done with sodium acetate buffer pH 6 affecting stability because it was nearer to neutral pH.

6.6.6 Percentage Transmittance Measurement

The value of % transmittance of 10, 50, 100 times diluted (Sodium acetate buffer pH 5.0) ME and MME was found to be nearer to 100 %, indicates that the system is optically clear which is a primary requirement for microemulsion.

Table 6.8 Percentage Transmittance for 100 times Diluted Microemulsion Systems

Sr. No.	Dilution Factor	% Transmittance	
		ME	MME
1	10 times	99.24 ± 0.15	98.64 ± 0.31
2	50 times	99.13 ± 0.24	98.23 ± 0.44
3	100 times	99.02 ± 0.34	97.69 ± 0.53

6.6.7 Conductance

Conductivity measurements provide a means of determining whether the diluted ME and MME shows oil as a continuous phase or water as a continuous phase. If the water phase is continuous then shows maximum conductance where in case of oil continuous phase the conductance is minimum. Conductivity value of optimized ME and MME formulation was found to be 74.21 ± 0.47 µS/cm and 79.24 ± 0.35 µS/cm.

6.6.8 Viscosity Measurement

Viscosity of the system depends on the type of the excipients used. The differentiation of low-viscous Newtonian from non-Newtonian flow is particularly important for undiluted ME and MME. Filling of very low-viscous formulations into suitable container can lead to loss of filling mass due to splashing around the dosing nozzle of the machine. This typically increases the rate of leaking as well as the weight variability of the units. ME was found to possess low viscosity and exhibited Newtonian behavior**.** However, the viscosity of the system gradually decreased with an increase in the aqueous phase.

Table 6.9 Viscosity of the ME and MME before and after Dilution

Sr. No.	Content	Viscosity (cP)	
		ME	MME
1	Initially	1012.31 ± 3.06 cP	1211.12 ± 2.71 cP
2	After 10 times dilution	101.44 ± 0.84 cP	131.65 ± 1.23 cP
3	After 100 times dilution	1.54 ± 1.37 cP	8.21 ± 1.03 cP

6.6.9 Cloud Point Measurement

The cloud point of optimized ME formulation was found to be 69 ± 2°C at which % transmittance was 73.51 ± 0.64 %, and for MME formulation it was found to be 67 ± 2°C at which % transmittance was 72.91 ± 0.54%. Therefore, it would suggest for both ME, a stable system formed after *in vivo* administration.

6.6.10 Assay

Assay of the ME and MME gives the amount of drug present in the formulation. Assay of the prepared ME and MME was found to be 99.14 ± 0.51 % and 99.15 ± 0.12 % respectively.

6.6.11 Histopathology Study

Both ME and MME were subjected to nasal toxicity study to evaluate the safety of components used in the formulation. The optical microscopy images of nasal mucosa treated with formulations are shown in Fig. 6.8.

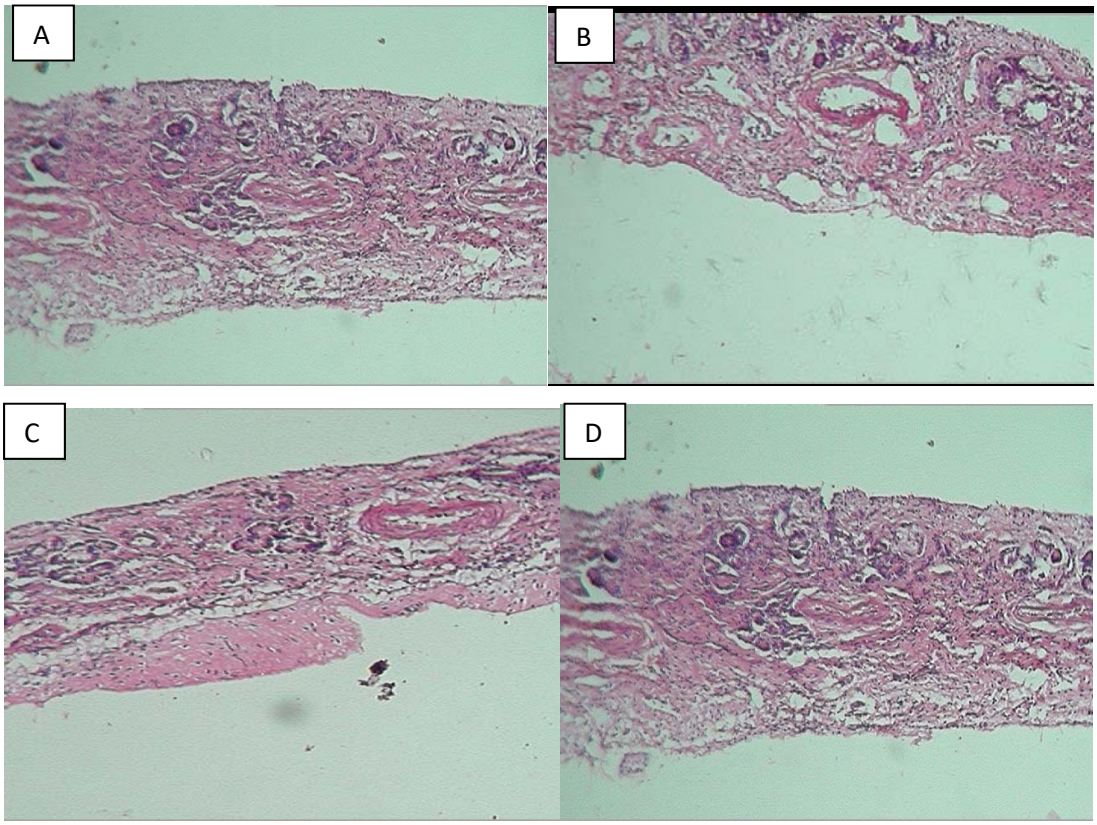

Fig. 6.8 Histopathology Study for Evaluation of Nasal Toxicity (A) PBS pH 6.4, (B) IPA, (C) ME, and (D) MME.

The nasal mucosa treated with PBS pH 6.4 (positive control) showed intact epithelial layer without any damage while mucosa treated with isopropyl alcohol (mucociliary toxic agent-Negative control) showed destruction of the epithelial layer and other nasal tissues.The nasal mucosa treated with ME and MME show no damage to the mucosal layer. Therefore, it can be predicted that prepared formulation seems to be safe for nasal administration.

6.6.12 TEM

Morphology of diluted ME was studied using TEM; image is shown in figure 6.9.The ME seems to have uniformly distributed, distinct clear spherical drug loaded globules. These image show that it has a uniform size and possess smooth surface morphology. No aggregation of the globules indicates the stability of the system.

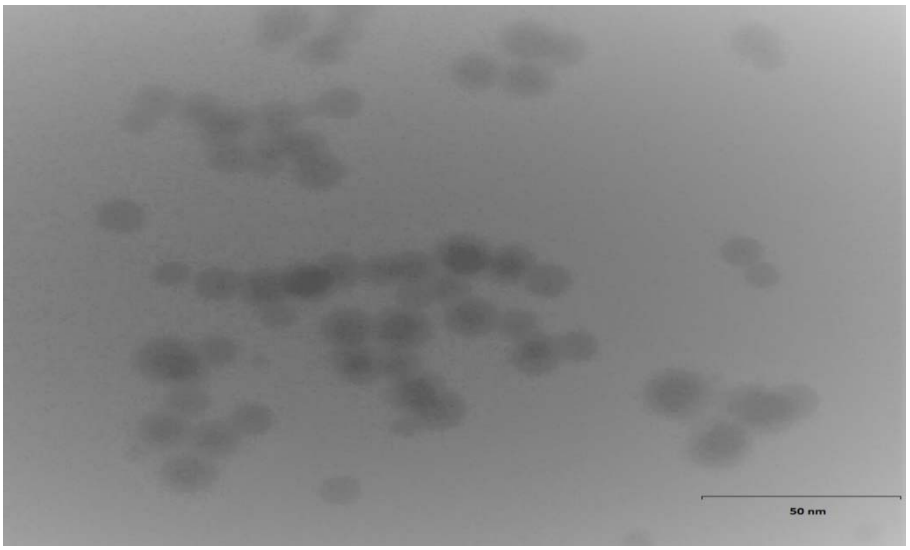

Fig. 6.9 TEM image of the Mucoadhesive Microemulsion of Vinpocetine

The globule size seemed to be in agreement with the result obtained from globule size analysis using Dynamic Light Scattering (DLS) method (Zetasizer).However, the actual particle size reported by TEM was found to be less than DLS. It can be postulated that these differences were shown by difference in size measurement methodology. The solvent layer attached to the particle; called hydrodynamic size is measured in DLS which is always greater than actual particle size measured by TEM.

6.7 References

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