Chapter 5 (Part- A)

Formulation and Development of oral SMEDDS

Management of Dyslexia and ADHD

5.1 Introduction

In recent years, majority of the drug candidates are hydrophobic in nature and associated with low bioavailability thus much attention has been focused on the system, which deals with the soluble active moiety in the formulated dosage form. Lipid-microemulsion formulations with particular emphasis on self-microemulsifying drug delivery systems (SMEDDS) or self-emulsifying drug delivery systems (SEDDS) to improve oral bioavailability of poorly water-soluble drugs specifically BCS class-II drug. (1, 2) SMEDDS are isotropic mixtures of oil, surfactants, and co-surfactants that form microemulsions when introduced into an aqueous phase under gentle agitation. Many researchers in various literature have reported the formulation techniques for SMEDDS. In any of the techniques, solubility of drug was determined in various vehicles (like Oil, Surfactant and Co-surfactant) and screened for emulsification efficiency. Ternary phase diagram is a very important tool to study the phase behavior of the SMEDDS and microemulsion system. It is represented in a triangular format, in which each coordinate represents one component of SMEDDS and/or microemulsion with 0 to 100% concentration in the increment of 10%. The systems, which solubilizes the highest amount of drug, has been selected and further studied for drug loading efficiency. D-optimal mixture experimental design is applied to optimize liquid SMEDDS using formulation variables; the oil phase X1, the surfactant X2 and the co-surfactant X3. The application of experimental mixture design has been demonstrated to be an efficient and satisfactory method for optimization of the formulation and to acquire the necessary information to understand the relationship between independent variables and dependent variables in a formulation. Experimental mixture design using Design-Expert® software was applied to optimize SMEDDS.

The aims of our present study was to formulate a self-microemulsifying drug delivery system (SMEDDS) for oral delivery of BCS Class-II drug, Modafinil, used for the management of disorder like ADHD and associated Dyslexia by enhancing memory. The formulation was optimized by using D-Optimal Mixture Design and was evaluated for its physico-chemical characterization, *in vitro* and *in vivo* potential.

5.2. Materials and Instruments

Alembic Pharmaceutical, Baroda, kindly gifted Modafinil. Tablet of Modafinil 100mg (Modvigil) was purchased from local pharmacy.

Table 5.1 List of Drug and Excipients

Sr. No.	Material	Name of Material	Manufacturer
1	Drug	Modafinil	Alembic Pharmaceutical, Baroda
2	Oil	Olive oil	S.D. Fine Chemicals, Mumbai, India
		Almond oil	S.D. Fine Chemicals, Mumbai, India
		Clove oil	S.D. Fine Chemicals, Mumbai, India
		Captex 200	Abitec Corporation, USA
		Capmul MCM C8	Abitech Corporation, USA
		Acconon CC	Abitec Corporation, USA
		Plurol Oleique CC 497	Gattefosse, France
		Labrafil K 2125 CS	Gattefosse, France
		Isopropyl Myistate	HiMedia Laboratories, Mumbai, India
3	Surfactant	Cremophor EL	BASF, Mumbai, India
		Tween 80	Spectrochem, Vadodara, India
		Tween 20	Spectrochem, Vadodara, India
		Labrasol	Gattefosse , France
		Labrafac PG	Gattefosse , France
		Lauroglycol 90	Gattefosse, France
		Brij 35	S.D. Fine Chemicals, Mumbai, India
		Kolliphore RH 40	S.D. Fine Chemicals, Mumbai, India
4	Co-surfactant	PEG-200	Spectrochem, Vadodara, India
		PEG-400	Spectrochem, Vadodara, India
		Propylene Glycol	Spectrochem, vadodara, India
		Glycerol	SD Fine Chemical Lab. Mumbai
		Transcutol HP	Fischer Scientific, Mumbai

Excipients used for formulation development are shown in Table 5.1. Equipments used at various stages are listed in Table 5.2. Purified HPLC grade water was obtained by filtering double-distilled water through nylon filter paper 0.45 μm pore size and 47 mm diameter, Purchase from Pall Life sciences, Mumbai, India.

Table 5.2 List of Equipments

Sr. No.	Equipment	Manufacturer
1	Digital Weighing Balance	Shimadzu, Japan
2	UV visible Spectrophotometer	UV-1600 Shimadzu, Japan
3	Magnetic stirrer	Remi equipments Pvt. Ltd., India
4	pH meter	Lab. India Pvt. Ltd, Mumbai
5	HPLC-UV detector	Shimadzu, Japan
6	Bath Sonicator	Remi equipments Pvt. Ltd, India
7	Filtration assembly	Drug Scientific Pvt. Ltd., Baroda
8	Centrifuge	Remi Instrument
9	Dissolution apparatus	Veevo, India
10	Transmission electron microscopy	Tecnai 20 Philips
11	FT-IR spectrophotometer	Bruker, Japan
12	Distillation assembly	Durga Glassware, India
13	Differential scanning calorimeter (DSC 60)	Shimadzu, Japan
14	Particle size analyzer (Malvern Zetasizer)	Malvern Instrument, UK
15	Transmission electron microscopy	JEM – 1011, Jeol, USA
16	Vortex mixer	Spinix, Japan
17	Incubator themoshaker	Bombay Lab. Services, Mumbai
18	Brookfield Digital viscometer	DV-1, Prime, USA

5.3 Preformulation Studies

Every drug has intrinsic chemical and physical properties which has been considered before development of pharmaceutical formulation. This study provide the framework for drug's combination with pharmaceutical ingredients in the fabrication of dosage form. (3) Objective of this study was to develop an elegant (stable, effective and safe) dosage form by establishing kinetic rate profile, compatibility with the other ingredients & establish physico-chemical parameter of new drug substance. From all these properties, drug solubility, partition coefficient, dissolution rate and stability play important role in preformulation study. Preformulation study is a step forward to save the time, give an idea about disastrous effect of formulation. Moreover it helpful in collection of information about the drug substance as well as excipients which may useful in the development of the stable formulation. Preformulation examinations are designed to recognize the physico-chemical properties as well as excipients that may affect the method of manufacture, formulation design and pharmacokinetic properties of the resulting product.

5.3.1 Characterization of Drug

5.3.1.1 Organoleptic Characterization

Modafinil was checked visually for organoleptic characteristics like color and odor of drug. It was examined for any unpleasant smell or change in color.

5.3.1.2 Melting Point Determination

Melting point was determined by adding small amount of drug Modafinil in a capillary tube sealed at one end. The capillary tube was placed in an electrically operated melting point apparatus in which temperature was gradually increased and the temperature at which the drug melt was recorded. Identification of drug was confirmed by comparing obtained value with reported value of melting point for drug.

5.3.1.3 FT-IR Study of Pure Drug

Potassium bromide (KBR) pellets was prepared with the addition of drug in the ratio of 1:100 and it was scanned using FT-IR instrument. The resultant spectra of drug shown in Fig. 5.1 and was compared with that of standard spectrum.

5.3.1.4 Thermal Behavior of Drug by DSC

Differential scanning calorimetry (DSC) was performed using DSC-60 to study the thermal behavior of drug. Drug sample around 2-3 mg was weighed and sealed in aluminum pan by applying external pressure. This aluminum pan was heated from 25°C to 300°C at a scanning rate of 10°C/min under nitrogen flow rate of 40 mL/min to create inert environment and to avoid oxidation due to presence of oxygen. The reference spectrum was used to compare the spectra of drug.

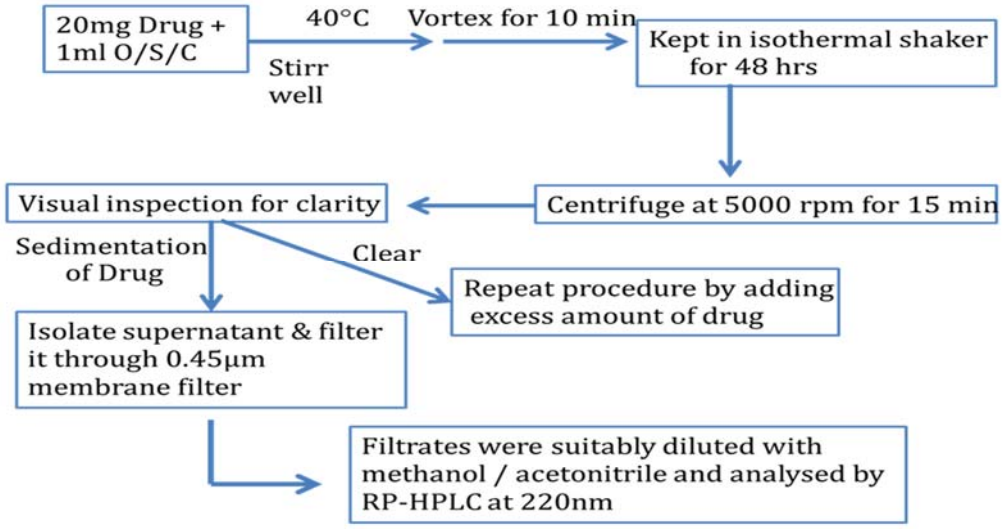
5.3.2 Screening of Excipients Based on the Solubility Study

Preparation of pseudo ternary phase diagram is an essential step to determine the quantity of components to formulate SMEDDS, but prior to that, selection of appropriate excipients to form stable drug loaded SMEDDS is a primary requirement. The important criterion for screening of components is solubility of drug in oils, surfactants and co-surfactants. (4, 5) To developed a stable oral formulation of SMEDDS, solubility of drug in oils is more important as the ability of SMEDDS to maintain the drug in solubilize form is greatly influenced by the solubility of the drug in oil phase. Emulsification efficiency was

determined to check whether the selected components for SMEDDS were able to formulate isotropically clear microemulsion on the dilution. The final selection of the components was based on the emulsifying ability of surfactant to emulsify the selected oil phase.

5.3.2.1 Solubility Determination

The solubility of Modafinil in various oils, surfactants and co-surfactants was determined by adding an excess amount of drug in each of oil, surfactant or co-surfactant in a glass vial, followed by gentle heating at 40°C in water bath for solubilization. The mixture was vortexed for 10 minutes using cyclomixer for proper mixing and mixture was kept in isothermal shaker for 48 hours at 37°C to attain equilibrium. The equilibrated samples were then centrifuged at 5000 rpm for 15min. (3) If sample remained clear without settling of drug, then same process was repeated by further adding of drug until it ascertains its maximum solubility. After further added quantity of drug was unable to dissolve, centrifugation was done and clear supernatant was isolated, diluted it with suitable solvent methanol/acetonitrile followed by filtration through 0.45 µm membrane filter. (6, 7) The concentration of Modafinil was determined in oils, surfactants or co-surfactants using HPLC. All studies were repeated thrice, with similar observations being made between the repeats. Results are represented in Table 5.6, 5.7, 5.8 and Fig. 5.3, 5.4, 5.5 for oil, surfactant and co-surfactant respectively. Outline of solubility determination is described in chart.



5.3.2.2 Screening of Excipients based on the Emulsification Efficiency

The emulsifying ability was evaluated by measuring the % transmittance (% T) of the mixture primarily prepared with oil and surfactant/co-surfactant. Results are represented in Table 5.9.

5.3.3 Drug Excipients Compatibility Study using FT-IR

Analysis of pure drug, isotropic mixture of excipients selected based on solubility study and blank mixture were carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra in order to remove the influence of residual moisture. For each of the spectrum, 8 scans were obtained at a resolution of 4 cm^{-1} from a frequency range of 4000–400 cm^{-1}. The result of spectra of Modafinil, blank SMEDDS, Modafinil loaded SMEDDS are shown in Fig.5.1, 5.6 and 5.7 respectively. From the data of the solubility study and emulsification study oil, surfactant and co-surfactant were selected and IR spectra of drug with the mixture of excipients were compared for the compatibility.

5.3.4 Screening of Surfactant: Co-surfactant Ratio based on Pseudo ternary Phase Diagram

The first step towards the formulation development was to determine the feasibility of the microemulsion formation. The boundaries of the microemulsion domains were determined by plotting pseudo ternary phase diagrams for the components selected from solubility studies. The pseudo ternary phase diagram of oil, surfactant and co-surfactant mixture with doubled distilled water was plotted using water titration method. Pseudo ternary phase diagrams were constructed in order to obtain the concentration range of components for the existing region of microemulsions. (7-9)

The weight ratio of surfactant to co-surfactant was varied as 1:3, 1:2, 1:1, 2:1 and 3:1. For each pseudo ternary phase diagram at a specific surfactant/co-surfactant (Smix) weight ratio, oil was added to the Smix at 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 respectively with constant stirring. (9, 10) To the resultant mixtures, water was added drop wise under vigorous stirring till the first sign of turbidity was observed visually in order to identify the end point. No heating was conducted during the preparation. Phase diagrams

were constructed using Chemix software. The area of the monophasic region was used as a tool for the selection of suitable surfactant and co-surfactant mixture. Based on the solubility study, oil (Clove oil), surfactant (Tween 80) and co-surfactant (PEG 400) were selected and phase diagram was constructed as represented in Fig. 5.8.

5.3.5 Optimization of Smix Ratio by Maximum Drug loading Capacity

To determine maximum drug loading capacity of the SMEDDS, excess quantity of drug was added in the formulation (mixture of oil and Smix, 1:9) with continuous stirring. After its saturation concentration, it was analyzed on the basis of stability on the dilution, particle size, PDI, % T and drug content and results are reported in Table: 5.10.

5.4 Formulation of SMEDDS

Based on ternary phase diagram, the optimum Smix ratio was selected and the drug loaded SMEEDS were prepared by dissolving the drug in oil. (9) Smix was added in to it with continuous stirring. The formulation was equilibrated at ambient temperature for at least 24 hrs, and was examined for sign of turbidity or phase separation prior to analysis.

5.5 Optimization of SMEDDS by D-Optimal Mixture Design

Quantitative aspects of effects and relationships among various formulation variables can be studied by incorporating batch design in the optimization process. Finding the optimum ratio of the selected components has traditionally involved changing the amount of one component at a time (one-factor-at-a-time approach). Although this approach can reveal the effects of individual components on the SMEDDS properties, it is resource intensive, interactions between components can not be investigated and the optimum formulation may not be found. (11) The development of SMEDDS can be made more efficient by using experimental design. In this approach, multiple variables that may affect the formulation properties are changed simultaneously in a strategic way, resulting in fewer experiments, interactions between variables being identified and quantified by the response parameters for the optimization of the formulation. In SMEDDS, stability of the dosage unit depends on the concentration of each ingredient where minor changes in their concentration leads to drastic change in the formulation. Here, in this system, total sum of the percentage of all the three ingredients is 100%. If the percentage of one component is increased, then it will

directly affect the percentage of one or more of the other components. Based on this information, most suitable design for the experiment is D-Optimal Mixture design generated by using Design-Expert® 9.0 software for three component system to conduct the optimization study. (12) SMEDDS components were selected based on the results of phase diagram and self-emulsification test.

This experimental design was used as a statistical tool to quantify relation between critical formulation variables and measured response for optimization. Based on the preliminary screening studies of SMEDDS components, X1, X2 and X3 factors were identified as key factors responsible for the change in the SMEDDS shown in Table 5.3.

X1, X2 and X3 were taken in three levels and their minimum as well as maximum concentration was determined in the preliminary trial, from which design was created by the software and it was characterized for specified parameters. Many formulations were prepared on the basis of D-Optimal Mixture experimental design and were assessed for globule size (Y1) and % Transmittance (Y2) after 1:10 times dilution with aqueous media, their desired criteria discussed in the Table 5.3.

Table 5.3 Variables with Desired Criteria of Dependable Variable

Sr. No.	Independent Variables		
1	X1	Amount of Oil	
2	X2	Amount of S-Mix	
3	X3	Amount of Aqueous Phase	
	Dependable Variable		**Criteria**
1	Y1	% Transmittance	Maximum (100%)
2	Y2	Globule Size (nm)	Minimum (< 50 nm)

5.5.1 Optimization of Formulation

The formulation was optimized by D-optimal mixture design using Design Expert 9.0 software. The 3 factors to be optimized were amount of oil, surfactant and co-surfactant. Runs were evaluated for the response variables and response values were subjected to multiple regression analysis to find out the relationship between the factors used and the response values obtained.

5.5.2 Statistical Analysis

Range of globule size and % Transmittance were obtained for all runs. The effect of formulation variables was statistically evaluated by applying one-way ANOVA at 0.05 level. The design was evaluated by quadratic model, which bears the form of equation:

$$\mathbf{Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \text{ (main effect)}}$$

$$\mathbf{+ \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \text{ (interaction effect)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ (eq.5.1)}}$$

Where Y is the response variable, β_0 the constant, β_1, β_2……,β_{33} are the regression coefficients. (11) X_1, X_2, X_3 stand for the main effect, X_1X_2…..,$X_1X_2X_3$ are the interaction terms, show how response changes when two factors are simultaneously changed. Using the regression coefficient of the factors, the polynomial equation for the response was constructed. Significantly contributing factors were only considered for equation generation.

Where Y corresponds to predicted response, X1 X2 and X3 correspond to the studied factors, $\beta_0$represent an intercept, and β_1– β_5 regression coefficient. F-test was applied to evaluate lack of fit within each model to identify best fitting model. The graphs were plotted for two responses. Using the regression coefficient of the factors, the polynomial equation for the response was constructed. Significantly contributing factors were only considered for equation generation.

After generating the polynomial equations relating the dependent and independent variables, the process was optimized for the responses Y1 and Y2 values. Optimization was performed to obtain the levels of independent variables, which would be directed for the minimum value of Y1 while keeping maximum value of Y2.

5.6 Result and Discussion

5.6.1 Organoleptic Characteristics

Preliminary study of Modafinil show white and odorless crystalline powder. Thus, visual identification of drug was confirmed that it did not show any sign of physical impurity.

5.6.2 Melting Point Determination

Melting point of Modafinil was found to be in the range of 166-168°C that can be correlated with the standard reportedvalue164-168 °C. Melting point is an identical characteristic to evaluate any compound for its purity. Any drastic change in the melting point of the drug indicates presence of impurity in the material. Therefore, it can conclude that drug was in the pure form.

5.6.3 FT-IR Study of Pure Drug

The characteristic absorption peaks of Modafinil were obtained from FT-IR spectrum study. The characteristic functional groups of the drug along with their wavenumbers in cm^{-1} shown in Table5.4. The FTIR spectrum of Modafinil shown in Fig. 5.1.

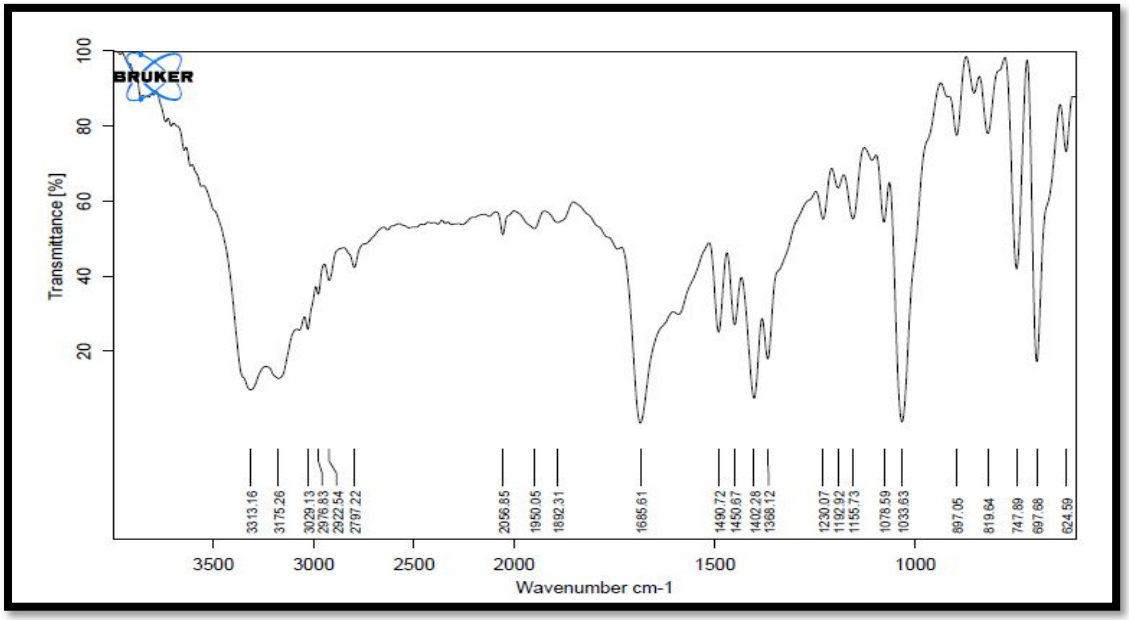


Fig. 5.1 FT-IR Spectra of Modafinil Drug

Table 5.4 Characteristic Peaks of FT-IR Spectra of Modafinil

Sr. No.	Functional Group	Wavenumber	
		Observed	Reported
1	Primary Amide Stretching	3313.16 cm^{-1}	3500-3300 cm^{-1}
2	C=O Stretching in amide	1685.61 cm^{-1}	1680-1630 cm^{-1}
3	Sulfoxide Stretching	1033.63 cm^{-1}	1030-1060 cm^{-1}
4	C-S Stretching	697.68 cm^{-1}	710-570 cm^{-1}
5	Methylene Stretching	2922.54 cm^{-1}	3000-2850 cm^{-1}
6	Aromatic Ring	1230.07, 1192.92, 1155.73 cm^{-1}	Region for stretching vibrations in the aromatic ring.

From above results, it was found that all characteristic peaks of function group were present in the drug. Result confirmed drug was Modafinil without any traces of impurity.

5.6.4 Thermal Behaviour of Drug by DSC

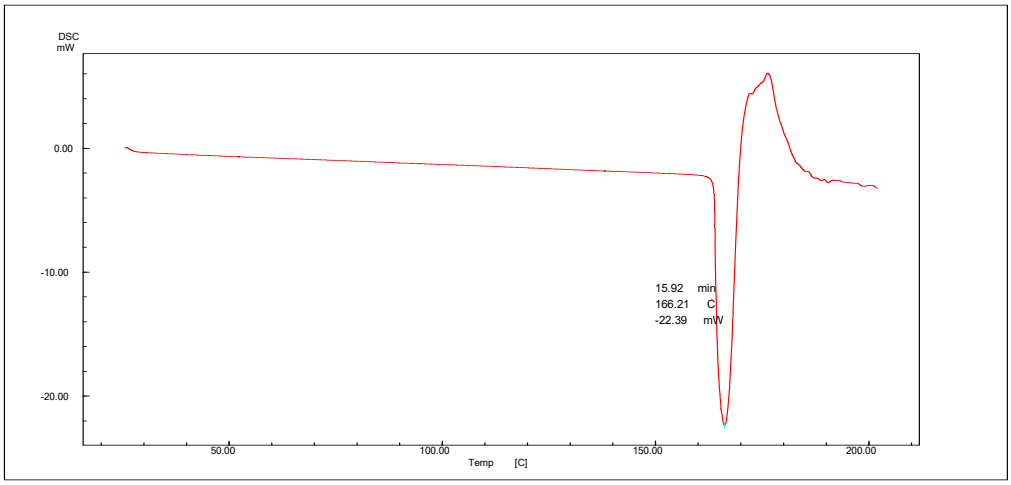


Fig. 5.2 DSC Thermogram of Drug: Modafinil

Table 5.5 Melting Point of Modafinil using Different Methods

Melting Point		
Reported	**Capillary Method**	**DSC**
164-168 °C	166-168 °C	166.21 °C

Fig. 5.2 represents DSC thermogram of drug, showing sharp endothermic peak and no other degradation peaks, devoid of any solvent peak and moisture peak. It was concluded that given sample was pure without any impurity. This result was supported by the data obtained from capillary method and reported value and are represented in Table 5.5.

5.6.5 Screening Excipients based on Solubility Study

Solubility of modafinil in different oils, surfactants and co-surfactants is given in Fig. 5.3, 5.4 and 5.5 respectively and the results are expressed as mean ± S.D. (n=3). The components used in the system should have high solubilization capacity for the drug, ensuring solubilization of drug in the resultant dispersion after dilution. Therefore, solubility of drug should be one of the main criteria for selection of oil, surfactant and co-surfactant. Another factor which can be affected by solubility is partitioning effect. If drug is not soluble and stable in mixture it will be diffused towards water at the time of formation of microemulsion and as drug is water insoluble and it will precipitate out in the formulation. Therefore, the selection of excipients is crucial factor for successful formulation.

Selection of Oil

Drug loading potential of the formulation was determined on the basis of the solubility data. If solubility of the drug is high in the oil, it may lead to lower the concentration of surfactant and co-surfactant/co-solvent, which minimizes the toxic effect of the surfactant. As shown in Table 5.6, maximum solubility of modafinil was found to be in the Clove oil (56.76±1.13mg/ml) followed by Capmul MCM (23.29±0.94mg/ml). Modafinil was found to have very low solubility in other oils. Clove oil is also reported to have useful emulsifying properties and it is classified in the GRAS (generally regarded as safe) component. Thus, the Clove oil was chosen as one of the component of the system.

Table 5.6 Solubility of Modafinil in Different Oils

Sr. No.	Oil	Solubility (mg/ml) *
1	Almond oil	3.81±0.61
2	Acconon CC	17.66±.82
4	Capmul MCM C8	23.29±0.94
5	Captex 200	8.69±0.54
6	Clove oil	56.76±1.13
7	Isopropyl Myistate	11.39±0.79
8	Labrafil K 2125 CS	17.93±0.905
9	Olive oil	12.94±0.74
10	Plurol Oleique CC 497	1.89±0.29

*Mean ± S.D. (n=3)

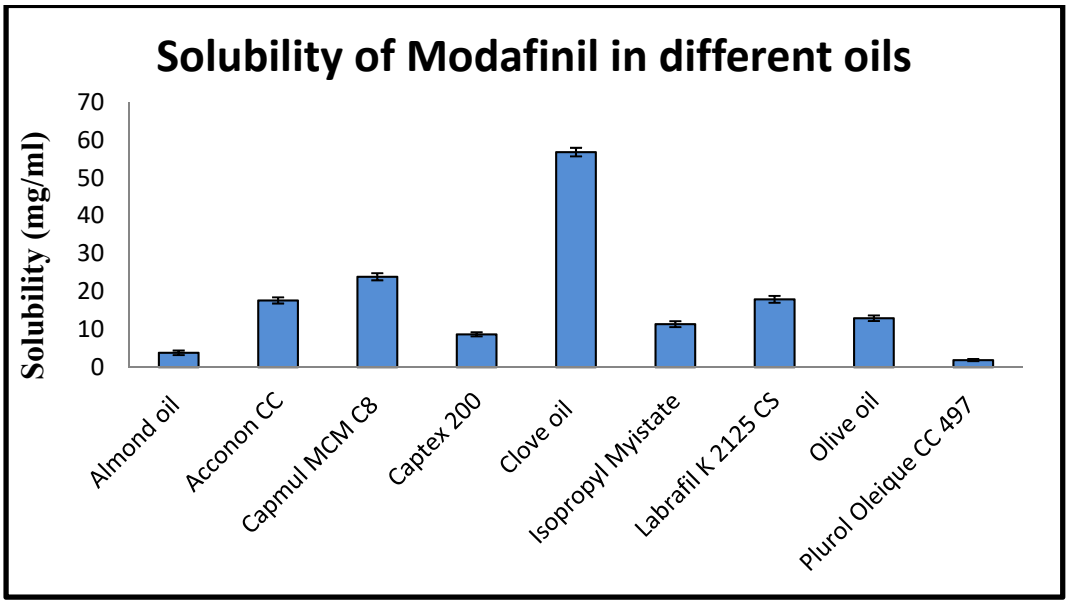


Fig. 5.3 Solubility of Modafinil in Different Oils

Selection of Surfactant

Different types of surfactants were studied to determine their ability to solubilize the drug. Non-ionic surfactants show better emulsion stability over a wide range of pH and ionic strength. They generally considered as a safer than ionic surfactants. Hence, different non-ionic surfactants having HLB in the range of (4-17) were investigated for stable o/w microemulsion. The Solubility of drug obtained for various surfactants are listed in Table 5.7, expressed as mean±S.D. (n=3).

Table 5.7 Solubility of Modafinil in Different Surfactants

Sr. No.	Surfactant	Solubility (mg/ml)*
1	Cremophor RH 40	8.65±0.41
2	Tween 80	20.89±0.68
3	Tween 20	20.34±0.52
4	Labrasol	14.26±0.69
5	Labrafac PG	11.41±0.93
6	Lauroglycol 90	13.58±0.41
7	Brij 35	3.40±1.13
8	Kolliphore RH 40	1.86±0.21

*Mean ± S.D. (n=3)

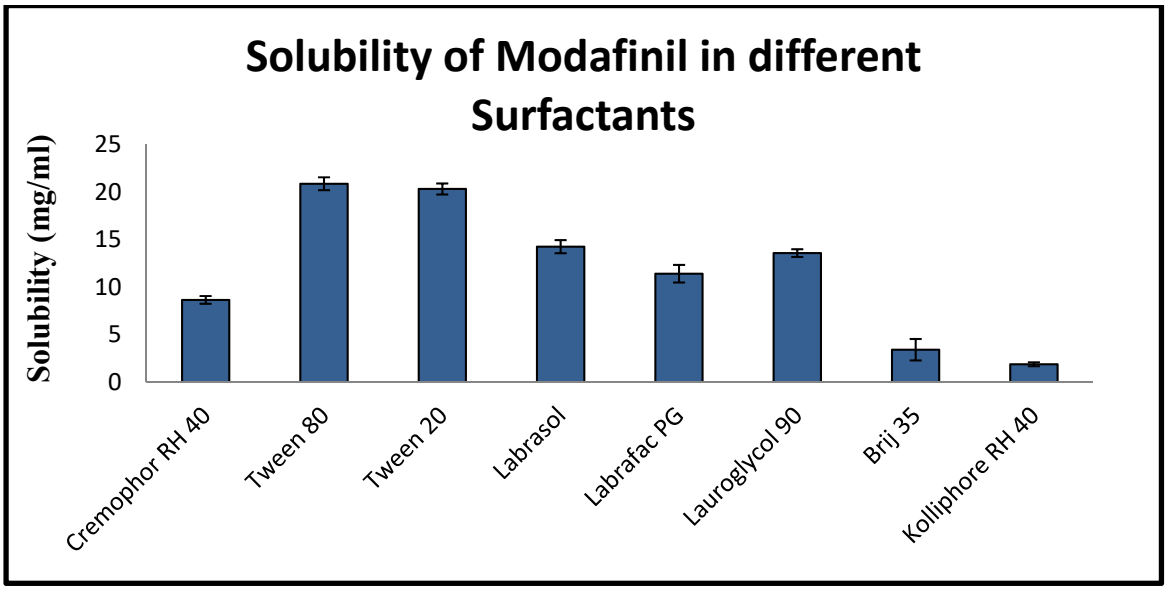


Fig. 5.4 Solubility of Modafinil in Different Surfactants

The solubility of the drug was found maximum in Tween 80 (20.89±0.68 mg/ml) and Tween 20 (20.34±0.52 mg/ml) followed by labrasol (14.26±0.69mg/ml). No significant difference was found in the solubility of the drug in Tween 80 and Tween 20. The final selection of the surfactant was dependent on the emulsifying ability of surfactant to emulsify the selected oil. It was evaluated by measuring the % transmittance of the prepared emulsion.

Selection of Co-surfactant

Different types of co-surfactants were studied to determine their ability to solubilize the drug. Co-surfactant helps surfactant for solubilization of drug as well as increased emulsifying efficiency of drug. Solubility of drug obtained for various co-surfactants are listed in Table 5.8.

Table 5.8 Solubility of Modafinil in Different Co-surfactants

Sr. No.	Co-Surfactant	Solubility (mg/ml) *
1	PEG-200	33.48±0.47
2	PEG-400	41.86±0.78
3	Propylene Glycol	15.34±0.32
4	Glycerol	08.69±0.30
5	Transcutol HP	19.81±0.84

*Mean ± S.D. (n=3)

Solubility of the drug was found maximum in polyethylene glycol (PEG) 400 (41.86±0.78 mg/ml) followed by PEG200 (33.48±0.47 mg/ml). Modafinil have less solubility in transcutol HP and propylene glycol than PEG. PEG 400 show more solubility than PEG 200. Therefore, for preparation of stable SMEDDS, PEG-400 was selected as a co-surfactant.

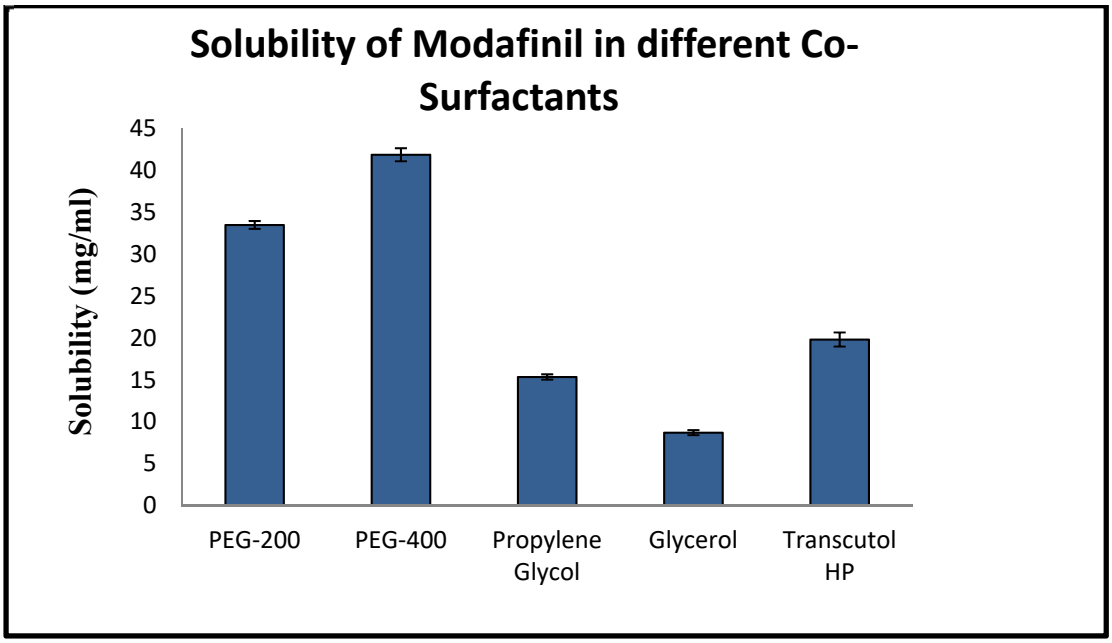


Fig. 5.5 Solubility of Modafinil in Different Co-surfactants

5.6.6 Screening of Excipients Based on Emulsification Efficiency

From the solubility study, clove oil and PEG-400 were confirmed for formulation of the SMEDDS as an oil and a co-surfactant respectively. The final selection of the surfactant was based on the emulsifying abilities to emulsify the selected oil phase; the emulsifying ability was determined by measuring % transmittance of the prepared emulsion. The % transmittance value of selected oil, clove oil with tween 80 and tween 20 are given in Table 5.9.

Table 5.9 Emulsification Efficiency

Sr. No.	Oil : Surfactant (1:2)	%Transmittance (at 650 nm)*
1	Clove oil : Tween 80	98.37 ± 0.19
2	Clove oil : Tween 20	91.18 ± 0.36

*mean±S.D. (n=3).

From the results of emulsification efficiency, it can be concluded that clove oil show highest transmittance with tween-80. Modafinil was found to have considerably good solubility in tween 80 and tween 20, but tween 80 show better result of transmittance and with PEG 400 it emphasis good result of particle size in the trial batches. Therefore, it was selected for further study.

5.6.7 Drug-Excipients Compatibility Studies

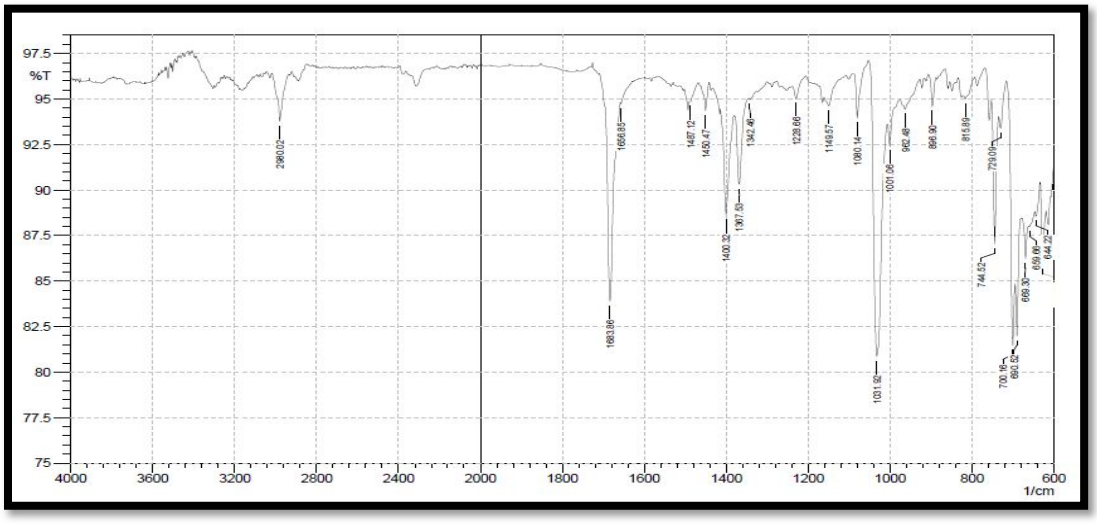


Fig. 5.6 FT-IR Spectra of Blank SMEDDS

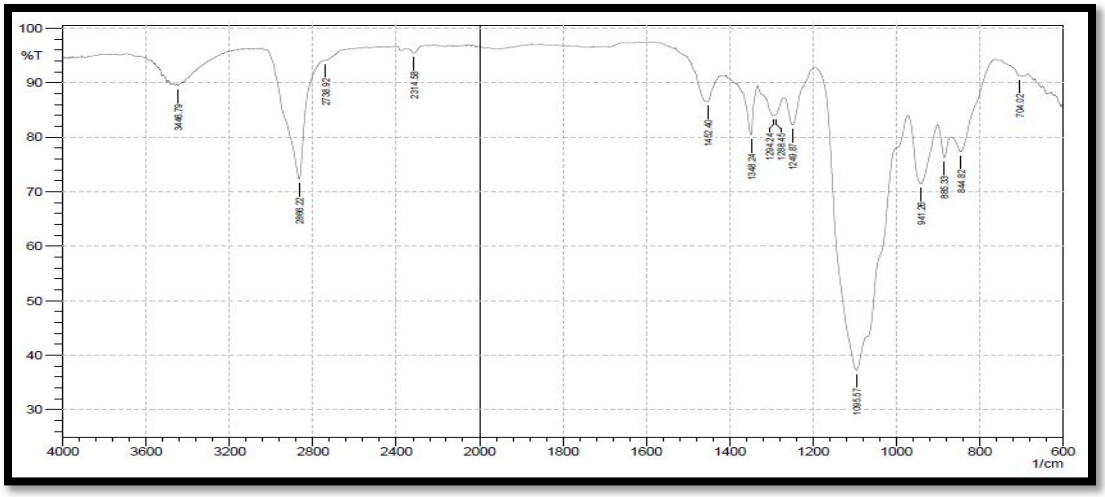


Fig. 5.7 FT-IR Spectra of Modafinil loaded SMEDDS

Fig. 5.1 and Table 5.4 shows IR spectrum of Modafinil, these characteristic peaks were also observed in Modafinil loaded SMEDDS (Fig. 5.7). Moreover, characteristic peaks of the individual excipients were also retained (Fig. 5.7) compared with blank SMEDDS (Fig. 5.6). From these results, it can be concluded that there was no interaction between Modafinil and excipients of the formulation.

5.6.8 Screening of Surfactant: Co-surfactant (Smix) Ratio based on Pseudo Ternary Phase Diagram

Pseudo ternary phase diagrams were constructed to identify the microemulsifying region. (13) Since surfactant and co-surfactant adsorb at interface and provide mechanical barrier to hinder coalescence, Smix ratio [S/Co-S (km)], plays an important role in stable microemulsion formation. Initially, maximum concentration of oil was taken, i.e. 90%, and amount of S/Co-S was kept to minimum, i.e., 10%. Gradually, oil concentration was decreased and that of S/Co-S was increased. It was observed that high concentration of oil forms a poor emulsion with very low entrapment efficiency of water and upon dilution with water it shows hazy appearance. The pseudo ternary phase diagrams were constructed at the ratios of S/CoS [Tween 80/PEG 400] (km) 3:1, 2:1, 1:1, 1:2 and 1:3, using clove oil.

Phase diagram preparation shows that concentration of oil was found to be a rate-limiting factor for stable SMEDDS preparation. At high concentration of oil, poor emulsion region was formed. The black region shows stable microemulsion region. At any point beyond this

boundary, microemulsion if formed initially, become turbid on further dilution of solution. This indicates formation of emulsion with higher particle size (>200 nm).

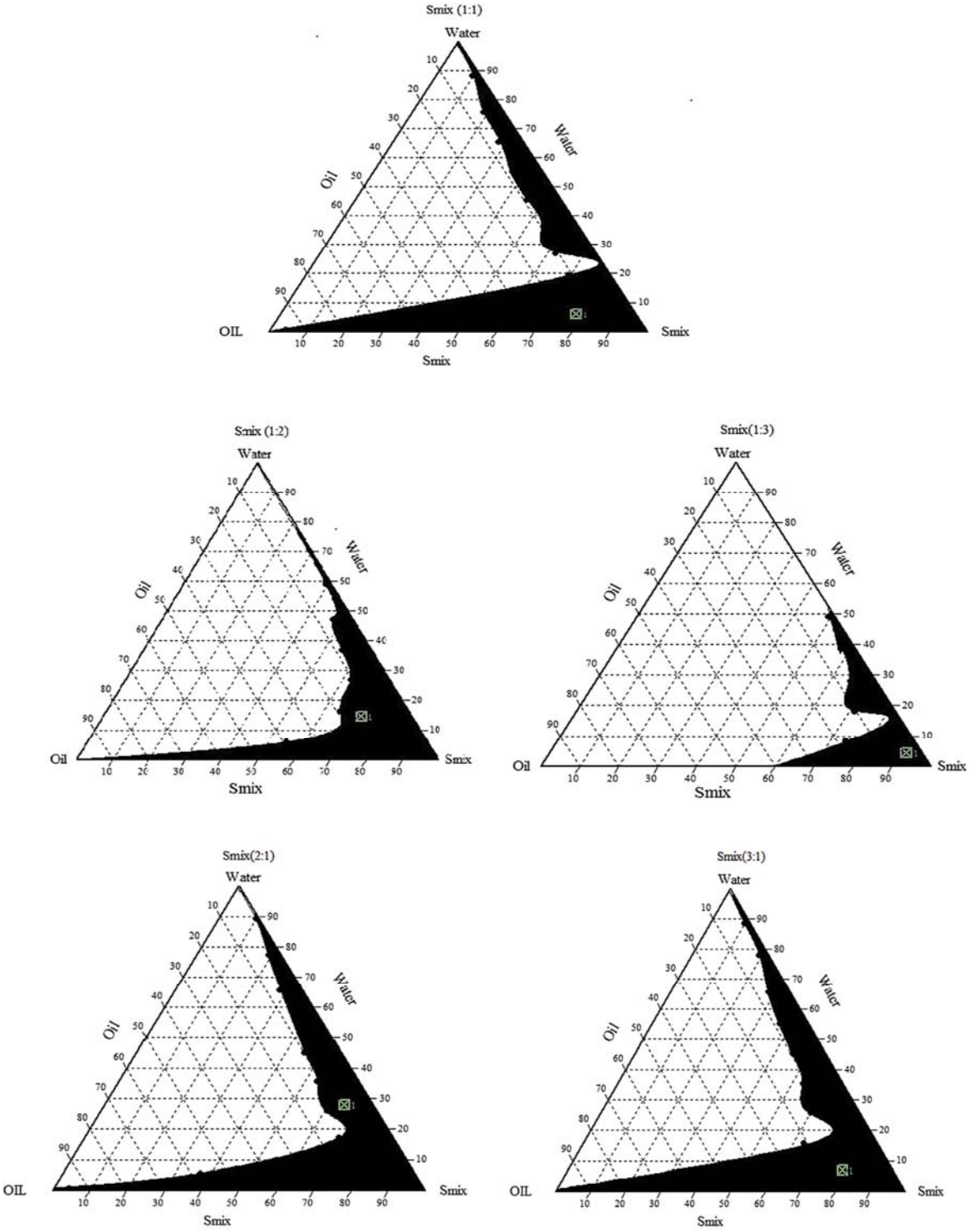


Fig. 5.8 Pseudo ternary Phase Diagram at Different Km Value

The pseudo ternary phase diagram (Fig.5.8) exhibits microemulsion region shaded with black colour. The Smix ratio at which system shows greater microemulsion region was selected for further study. It was observed that, as the ratio changes from 3:1 to finally 1:2, black region of microemulsion formulation decreases respectively. Thus, it was found that increased surfactant concentration results in good microemulsion region. However, higher concentration of surfactant (> 70 %) leads to gastric irritation and other side effects. Therefore, ratio of surfactants mixture was further investigated and optimized by experimental design which would help to find out minimum ratio of surfactant at which desired results would be obtained. Here, Km ratio 3:1 and 2:1 shows stable microemulsion formulation.

5.6.9 Optimization of Smix Ratio based on Maximum Drug Loading Capacity

Result of characterization after a maximum drug loading in different Smix/Km ratio are represented in Table 5.10. Km 1:1 was not selected for the drug loading study due to its instability in production of isotropically clear microemulsion on dilution. With ratio of 2:1 and 3:1, incorporation of 100 mg drug resulted in stable microemulsion after 4 hrs of 100 times dilution. On 100 times dilution, ratio of 2:1 solubilized 125mg of drug and was stable for 4 hrs; whereas with 3:1 ratio, 125mg of drug loading resulted in formation of microemulsion but after 4hrs precipitation of microemulsion occurred.

Table 5.10 Drug Loading Capacity in Different Smix/Km Ratio

Sr. No.	Km Ratio	Drug Loading (mg)/ 5 gm of SMEDDS	Particle Size (nm)	PDI	% Transmittance at 0 hr	Stability on dilution(100 times)	
						0 hr	After 4 hr
1	2:1	100	27.21	0.159	98.72±0.18	Stable	Stable
		125	**29.51**	**0.218**	**98.34±0.14**	**Stable**	**Stable**
		150	48.31	0.196	95.29±0.11	Stable	Precipitation
2	3:1	100	22.68	0.214	98.89±0.14	Stable	Stable
		125	39.34	0.286	97.46±0.09	Stable	Precipitation
		150	32	0.321	97.34±0.14	Stable	Precipitation

From the above data it can be concluded that Km 3:1 has low potential for drug solubilization compared to Km 2:1. Km 2:1 has more concentration of co-surfactant

compared to Km 3:1 and drug shows maximum solubility in co-surfactant (PEG-400, 41.86mg/ml) than surfactant (Tween 80, 20.89 mg/ml), therefore Km 2:1 can dissolve high quantity of drug, thus, Km 2:1 was selected for further optimization.

5.6.10 Finalization of Concentration Range for the SMEDDS Formulation

Based on the results of the solubility studies and emulsification efficiency, Clove oil was selected as oily carrier phase for the SMEDDS, Tween 80 and PEG-400 were selected as surfactant and co-surfactant respectively. From the different trial batches of SMEDDS maximum and minimum concentration of different excipients were calculated which are represented in Table 5.11.

Table 5.11 Concentration Range of the Ingredients from Preliminary Trial

Sr. No.	Class	Name of Excipients	Concentration Range
1	Drug	Modafinil	125 mg
2	Oil	Clove oil	8-14 %
3	Surfactant	Tween-80	56-65 %
4	Co-surfactant	PEG-400	25-35 %

5.6.11 Optimization of SMEDDS Using D-Optimal Mixture Design

Experimental designs were used as a systematic approach to simultaneously identify excipients and the ratio of the selected excipients. Furthermore, concentration of the excipients was selected based on (pseudo)ternary mixture designs, which is demonstrated in Table 5.12.

Table 5.12 Levels of Independent Factors in Experimental Design

Coded Values	Actual Values		
	X1: Oil (Clove oil)	**X2: S** (Tween 80)	**X3: CoS (**PEG 400)
Low Actual	8	56	65
High Actual	14	25	35

Statistical design was explored and checked suitability for exploring quadratic response surfaces and constructing polynomial models, which in turn was used in optimizing a process using a small number of experimental runs. In this design, three variables (oil, surfactant and co-surfactant concentration) were used to study the combined effect of them on the mean globule size (nm) and % Transmittance of the formulation; it is represented in

Table 5.13. 16 experiments were designed by the software with 5 center points. Dependent variables were mean globule size as Y1 and % Transmittance (%T) as Y2 were evaluated.

Table 5.13 D-Optimal Mixture Design for the Optimization of SMEDDS

Run	X1 (%)	X2 (%)	X3 (%)	Y1 (nm)	Y2 (%)
1	8.00	62.336	29.663	21.64	99.5
2	10.921	59.296	29.782	27.94	96.88
3	8.00	59.611	32.38	24.26	99.14
4	13.767	56.00	30.23	26.34	95.49
5	8.131	56.868	35.00	21.87	99.38
6	9.792	65.00	25.207	22.3	97.47
7	8.00	64.460	27.539	18.94	99.51
8	10.765	61.991	27.242	25.67	96.58
9	13.767	56.00	30.232	26.74	95.72
10	8.00	64.460	27.539	18.94	99.62
11	13.869	61.130	25.00	30.61	94.58
12	11.103	56.00	32.896	25.71	96.72
13	13.793	58.581	27.624	26.87	95.34
14	8.131	56.868	35.00	24.69	98.86
15	9.792	65.00	25.207	22.3	97.88
16	13.869	61.130	25.00	30.61	94.81

X1: Oil concentration (%w/w), X2: Surfactant concentration (%w/w), X3:Co-surfactant concentration (%w/w), Y1: Globule Size (nm), Y2: % Transmittance.

5.6.12 ANOVA Analysis for Response Y1: Globule size

Model selection for the globule size analysis was done by comparing F and P values for different models and whichever model show lesser F and P value was selected as the best suitable model.

From Table 5.14 it was confirmed that it follows Quadratic model as it was best suitable model. The mean globule size was selected as a response and was ranged between 18.94 to 30.61 nm, which indicate that the response was sensitive towards the studied factor.

Table 5.14 Selection of the Model for Globule Size Analysis (Statistical Analysis)

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	9772.8053	1	9772.8053			
Linear vs Mean	133.7776	2	66.8888	15.6576	0.0003	
Quadratic vs Linear	**44.2448**	**3**	**14.7482**	**13.0625**	**0.0009**	**Suggested**
Sp Cubic vs Quadratic	3.8527	1	3.8527	4.6620	0.0591	
Cubic vs Sp Cubic	0.8146	3	0.2715	0.2459	0.8614	
Residual	6.6231	6	1.1038			
Total	9962.1183	16	622.6323			

To identify the significant parameters and their interactions, analysis of variance was performed for each parameter. The values of the coefficients X1, X2 and X3 are related to the effect of these variables on the response. A positive sign of coefficient indicate a synergistic effect while a negative term indicates an antagonistic effect upon the response. Larger coefficient means the independent variable has more potent influence on the responses. Coefficients with P-value less than 0.05 had a significant effect on the measured response. ANOVA for the mean globule size is represented in Table 5.15.

Table 5.15 ANOVA Analysis of Experimental Design for Globule Size Analysis

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F	
Model	178.0224	5	35.6045	31.5349	< 0.0001	**Significant**
Linear Mixture	133.7776	2	66.8888	59.2434	< 0.0001	
AB	18.2439	1	18.244	16.1587	0.0024	
AC	1.5463	1	1.5463	1.3695	0.2690	
BC	8.0298	1	8.0298	7.1120	0.0236	
Residual	11.2905	10	1.1290			
Lack of Fit	7.2343	5	1.4468	1.7835	0.2704	**Not significant**
Pure Error	4.0562	5	0.81124			
Core Total	189.3129	15				

The Model F-value of 31.53 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case Linear Mixture Components, AB, BC are significant model terms. Values greater than 0.1 indicate the model terms are not significant.

Table 5.16 ANOVA Study Results for Globule Size Analysis

Sr. No.	Parameters	Results of Response (Y1)
1	Std. deviation	1.06257
2	Mean	24.7144
3	C.V.%	4.29939
4	Press	28.1631
5	R-squared	0.94036
6	Adjusted R-squared	0.91054
7	Predicted R-squared	0.85124
8	Adeq Precision	16.8675

If there are many insignificant model terms, model reduction may improve model. The "Lack of Fit F-value" of 1.78 implies the Lack of Fit is not significant relative to the pure error. There is a 27.04% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good.Therefore this model can be utilized for further analysis.

The "Pred R-Squared" of 0.8512 is in reasonable agreement with the "Adj R-Squared" of 0.9105. “Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here signal to noise ratio of 16.868 indicates an adequate signal. This model can be used to navigate the design space. ANOVA for the same is represented in Table 5.16.

5.6.12.1 Mathematical Model for Globule Size (Y1)

Actual Equation

Y1= -18.03501*X1-2.3899*X2-2.9907*X3+0.35241*X1*X2 +0.09263*X1*X3 +0.1113*X2*X3……..……. (eq. 5.2)

From Table 5.15 and from mathematical model for particle size, it was found that variables (X1, X2 and X3) and their interactions (X1X2, X2X3) had significant effect on response

Y1. Here X1 shows negative effect suggesting an inversely proportional relationship with the mean globule size. Here amount of Clove oil was found to have negative effect, suggesting an indirect proportional relationship with the mean globule size but its combination effect with surfactant and co-surfactant shows positive impact. It means that high concentration of Clove oil provides SMEDDS with large globule size in correlation with its combinatorial effect. Surfactant and co-surfactant shows antagonistic effect on the particle size.

5.6.12.2 Contour and 3D Surface Plot

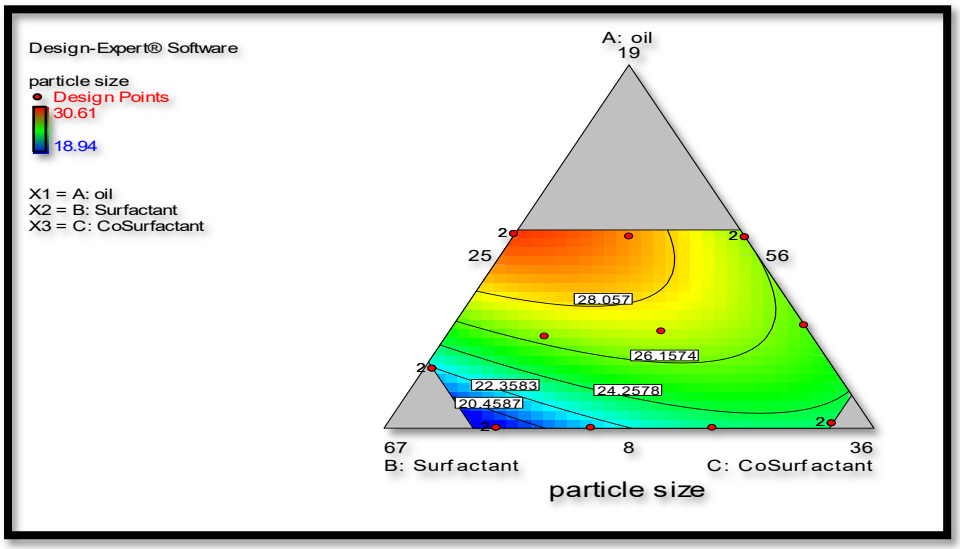


Fig. 5.9 Interaction Effects of Oil, Surfactant and Co-surfactant on Globule Size using Contour Plot

The relationship between the dependent and independent variables was elucidated using contour and 3D response surface plots (Fig. 5.9 and 5.10 respectively). The effect of X1, X2 on particle size Y1 represent in Fig. 5.9, it reveals that the size of oil globules would be smaller at low value of oil concentration. As the concentration of surfactant and co-surfactant increases, particle size decreases. The globule size of the emulsion is important factor in SMEDDS formulation, as reported in various literature this determines the rate and extent of drug release as well as *in vivo* absorption.

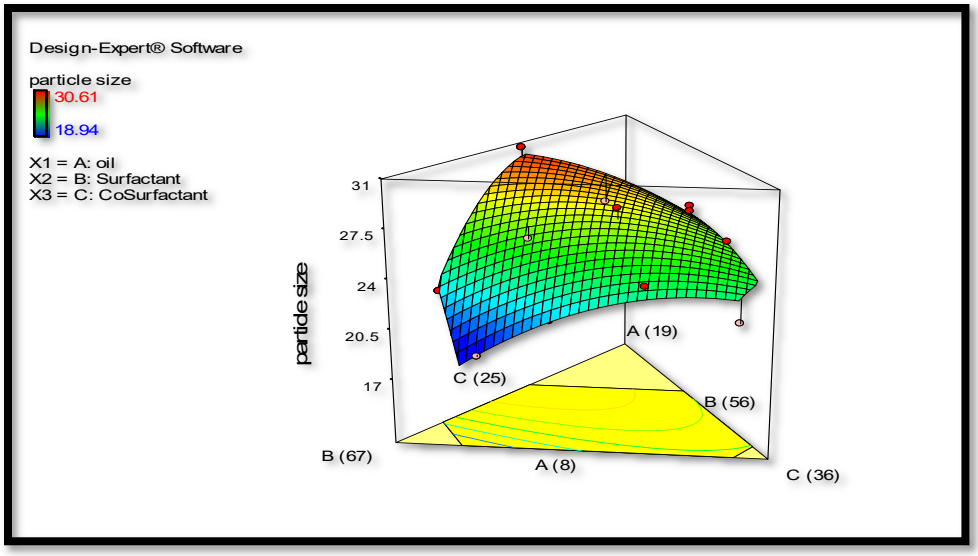


Fig. 5.10 3D Surface Plot: Interaction Effects of Oil, Surfactant and Co-surfactant on Globule Size using 3D Surface Plot

5.6.13 ANOVA Analysis for Response Y2: % Transmittance

Table 5.17 Selection of the Model for % Transmittance Analysis (Statistical Analysis)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	151652	1	151651.8			
Linear vs Mean	48.6445	2	24.32226	144.877448	< 0.0001	
Quadratic vs Linear	**1.70879**	**3**	**0.569597**	**12.02521324**	**0.0012**	**Suggested**
Sp Cubic vs Quadratic	0.03011	1	0.030114	0.61102647	0.4545	
Cubic vs Sp Cubic	0.15937	3	0.053123	1.12159375	0.4119	
Residual	0.28419	6	0.047364			
Total	151703	16	9481.416			

Model selection for % transmittance analysis was done by comparing F and P values for different models and whichever model shows lesser F and P value was selected as the best suitable model.

From Table 5.17 it can be confirmed that it follows Quadratic model. Lac of fit and model summary shows quadratic model as a best suitable model. % transmittance was selected as a response and was ranged between 94.58% - 99.72%, which indicates that the response was sensitive towards the studied factor. To identify the significant parameters and their interactions, analysis of variance was performed for each parameter. The values of the coefficients of X1, X2 and X3 are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response.

Larger Co-efficient means the independent variable has more potent influence on the responses. Coefficients with P-value less than 0.05 had a significant effect on the measured response. The ANOVA Table 5.18 for the same was mentioned underneath:

Table 5.18 ANOVA Analysis of Experimental Design for % Transmittance Analysis

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	**50.3533**	**5**	**10.07066**	**212.6096232**	**< 0.0001**	**Significant**
Linear Mixture	48.6445	2	24.32226	513.4862381	< 0.0001	
AB	1.19384	1	1.193842	25.2041249	0.0005	
AC	0.48171	1	0.481709	10.16974683	0.0097	
BC	0.0006	1	0.000601	0.012695688	0.9125	
Residual	0.47367	10	0.047367			
Lack of Fit	**0.19547**	**5**	**0.039094**	**0.702620826**	**0.6460**	**Not significant**
Pure Error	0.2782	5	0.05564			
Core Total	50.827	15				

The Model F-value of 212.61 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case Linear Mixture Components, AB, AC are significant model terms. Values greater than 0.1 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve model. The "Lack of Fit F-value" of 0.70 implies

the Lack of Fit is not significant relative to the pure error. There is a 64.60% chance that a "Lack of Fit F-value" this large could occur due to noise. So this model can be utilized for further analysis.

The "Pred R-Squared" of 0.9751 is in reasonable agreement with the "Adj R-Squared" of 0.9860. “Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here the signal to noiseratio of 36.7346 indicates an adequate signal. This model can be used to navigate the design space.

Table 5.19 ANOVA Study Results for % Transmittance Analysis

Sr. No.	Parameters	Results of Response (Y2)
1	Std. deviation	0.217639407
2	Mean	97.35625000
3	C.V.%	0.223549497
4	Press	1.264518615
5	R-squared	0.990680753
6	Adjusted R-squared	0.986021130
7	Predicted R-squared	0.975121112
8	Adeq Precision	36.73466389

5.6.13.1 Mathematical Model for % Transmittance (Y2)

From Table 5.19 and mathematical model for % transmittance, it was found that variables (X1, X2, X3) and their interactions (X1X2, X1X3) had significant effect on response Y2.

Actual Equation

$$Y2= 6.46417478*X1 +1.270098931*X2 +0.93124029*X3 -0.090148316*X1*X2 - 0.051705199*X1*X3 -0.000963353*X2*X3 \quad \text{(eq. 5.3)}$$

Here X1, amount of Clove oil was found to have positive effect, suggesting a direct proportional relationship with % Transmittance but its combination effect with surfactant and co-surfactant has negative impact. It means that high concentration of Clove oil provides SMEDDS with low % Transmittance in correlation with its combinatorial effect. Concentration of surfactant and co-surfactant shows direct proportional relationship with % Transmittance.

5.6.13.2 Contour and 3D Surface Plot

The relationship between the dependent and independent variables was elucidated using contour and 3D response surface plots (Fig. 5.11 and 5.12 respectively). The effect of X1,

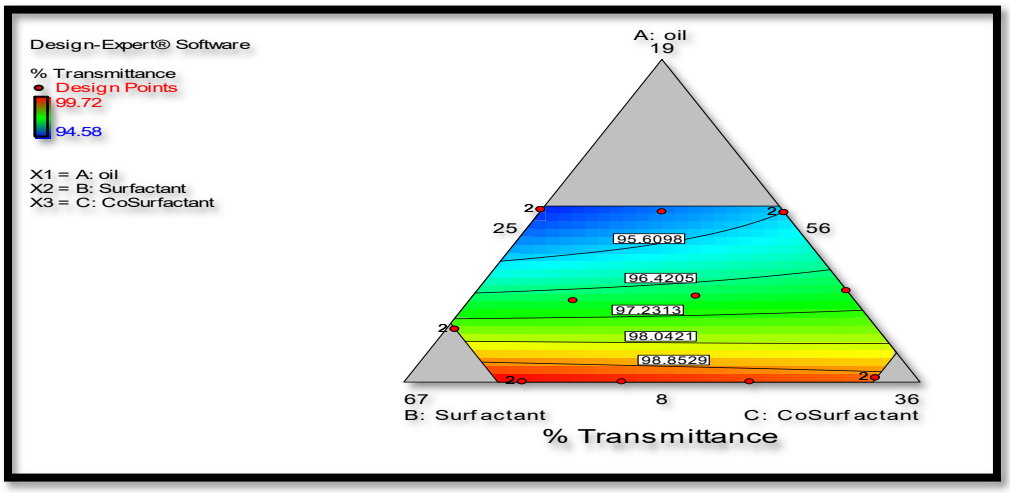


Fig. 5.11 Contour Plot: Interaction Effects of Oil, Surfactant and Co-surfactant on Percentage Transmittance

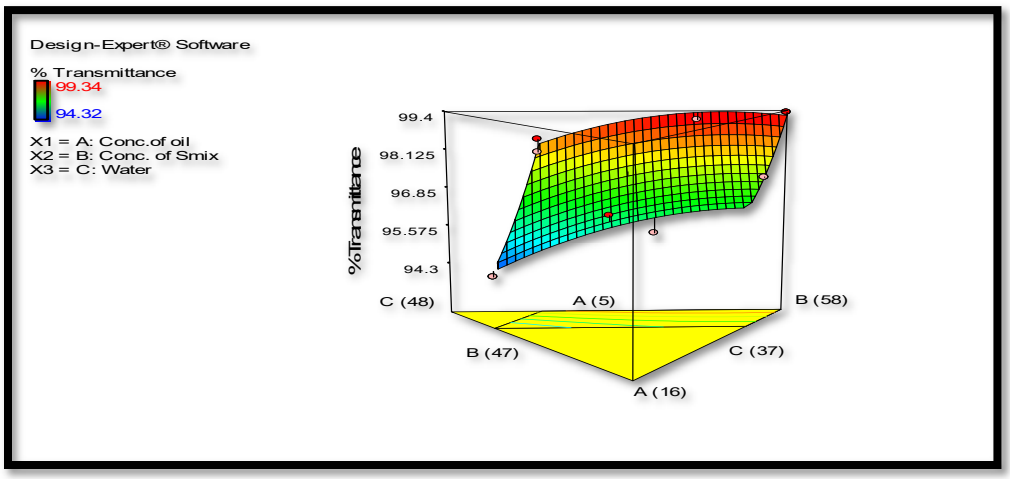


Fig. 5.12 3D Surface Plot: Interaction Effects of Oil, Surfactant and Co-surfactant on Percentage Transmittance

X2 and X3 on % Transmittance Y2 reveals that % Transmittance was increasing at low value of oil concentration. As the concentration of surfactant and co-surfactant increases it increases % Transmittance. % Transmittance of the emulsion is an important factor in SMEDDS formulation, as it determines stability of the formulation on *in vivo* application.

5.6.14 Numerical Optimization

Table 5.20 Constraints Applied for Selection of Optimized Batch

Name	Goal	Lower Limit(%w/w)	Upper Limit(%w/w)
A:oil	In range	8.0	10.0
B: Surfactant	In range	60.0	65.0
C:Co-surfactant	In range	25.0	30.0
Globule size	Minimize	18.0	22.0
%Transmittance	Maximize	95.0	100.0

Table 5.21 Formulation Parameters Based On Desirability

Oil	Surfactant	Co-surfactant	%Transmittance	Size(nm)	Desirability
8.00	65.00	27.00	99.675	18.253	0.984

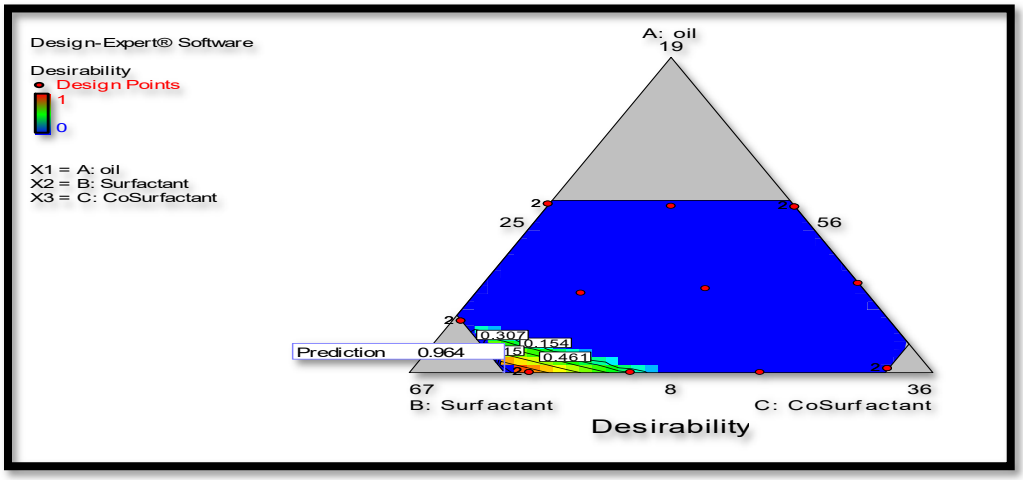


Fig. 5.13 Desirability Plot for Selection of Parameters

Numerical optimization i.e. desirability study for the optimization of SMEDDS was obtained by lower and upper limit of oil, surfactant, co-surfactant and with desired criteria limit of globule size and transmittance (Table 5.20). Formulation optimization was based on the desirability, which may range from 0 to 1. Table 5.21 and Fig. 5.13 represents the desirability batch generated by software with desirability near to maximum one; this batch was prepared and evaluated by experimentally for confirmation and optimization of Modafinil SMEDDS and result was tabulated in Table 5.22. The optimum composition was selected on the basis of desirability plot (Fig. 5.13). The factor A, B and C provided optimum response respectively at 8 % w/w, 65 % w/w and 27 % w/w with 18.253 nm of globule size and 99.675% transmittance with desirability 0.964.

Table 5.22 Suitability of Predicted Desirability Plot for Optimized Formulation

Independent Variables	Predicted Values	Desirability
Oil	8.00	0.964
S- Mix	65.00	
Aqueous	27.00	
Response	**Predicted**	**Actual**
% Transmittance	99.675	99.515±0.19
Size (nm)	18.253	19.210±0.47

T test values for both factors were lower than their T standard values. So, there was no significant difference ($p>0.05$) between predicted and experimental values of both the factors. Therefore, the model was validated.

5.6.15 Graphical Optimization

Graphical Optimization by Overlay Plot for predicted design space; with input lower and upper limit of transmittance and size; graph of overlay plot was generated by software and is represented in Fig.14 from the different batches generated, two batches were evaluated by experimentally and results were tabulated in Table 5.23.

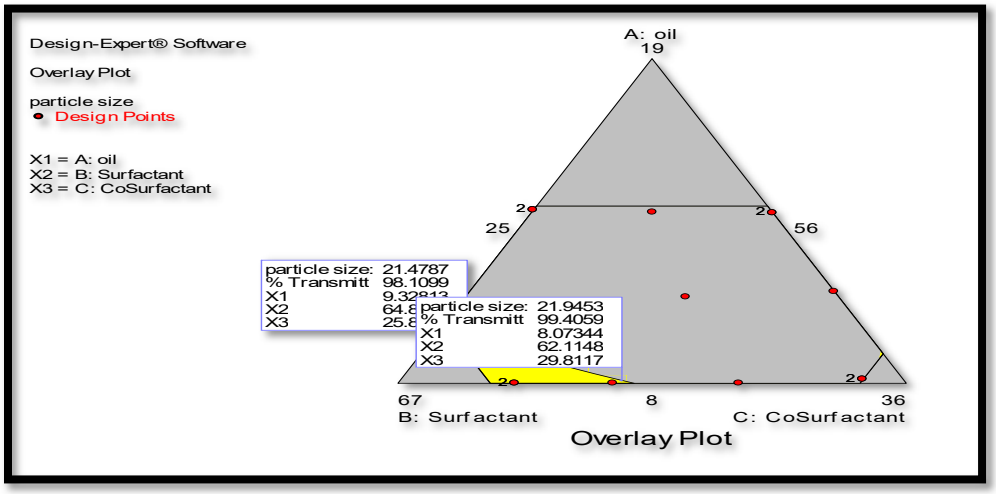


Fig. 5.14 Overlay Plot for SMEDDS

Experimental design was used for multiple responses Y1: Globule size and Y2: % Transmittance, it is necessary to obtain a region that provides optimum values of factors. Overlay plot (Fig. 5.14) can be obtained by superimposing contour plots of responses Y1

and Y2, which displays the area of feasible response values in the factor space. The region highlighted in yellow color is the area in which a slight variation in the critical variables won't affect the final response and the response will be in desired range. Regions that do not fit the optimization criteria are shaded gray while design space which is accepted are colored yellow. Flags placed in overlay plot shows predicted values of desired response with optimized values of variables.

Table 5.23 represent that T test values for both factors and for both the batches were lower than their T standard values. There was no significant difference between predicted and experimental values of both the factors. Therefore, the model was validated. The selected batch generated by software was same as shown in desirability plot.

Table 5.23 Predicted Batch of Analysis for the SMEDDS within Desirable Area

Independent Variables	**Predicted Values**	
Oil	08.0734	
Surfactant	62.1148	
Co surfactant	29.8117	
Response	**Predicted**	**Actual**
% Transmittance	99.4059	99.50±0.501
Size (nm)	21.9453	21.84±0.773
Oil	09.3281	
Surfactant	64.8513	
Co surfactant	25.8325	
Response	**Predicted**	**Actual**
% Transmittance	98.109	98.34±0.168
Size (nm)	21.478	21.02±0.712

Table 5.24 Concentration of the Ingredients for Optimized Batch

Sr. No.	**Component**	**Optimized concentration**
1	Drug: Modafinil	125 mg
2	Oil: Clove oil	8 %
3	Surfactant: Tween-80	65%
4	Co-surfactant: PEG-400	27%

The optimised batch was prepared by using the formula denoted in Table 5.24 and used for the physical, chemical and biological evaluation.

Chapter 5 (Part- B)

Formulation and Development of NTB Microemulsion

Management of Dyslexia and ADHD

5.7 Introduction

The selection of an appropriate dosage form is a critical step because a dosage form with poor drug delivery or absorption can make a useful drug worthless. There are various approaches in delivering a therapeutic substance to the target site in a controlled release fashion. One such approach is using microemulsion as carriers for drugs.(14) Interest in these versatile carriers is increasing and their applications have been diversifying to various administration routes in addition to the conventional oral route. This can be attributed to their unique solubilization properties and thermodynamic stability which has drawn attention for their use as carrier for drug targeting to the brain. Intranasal drug delivery is one of the focused delivery options for brain targeting, as it is noninvasive, painless delivery and it does not require sterile preparation. Brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation. Lipophilic drug delivery by intra nasal route can easily cross BBB by travelling through transcellular pathway. (14, 15) Microemulsions are isotropic systems, which are difficult to formulate than ordinary emulsions because their formulation is a highly specific process involving spontaneous interactions among the constituent molecules. These systems are broadly categorized into three types: (1) oil-in-water (o/w) microemulsions, (2) water-in-oil (w/o) microemulsions and (3) bicontinuous microemulsions. Many researchers in various literatures have reported the formulation techniques for microemulsions. These techniques are mainly pseudo ternary diagram construction and titration method. (16) Ternary phase diagram is a very important tool to study the phase behavior of the microemulsion system. Ternary phase diagram can be represented in a triangular format, in which each coordinate represents one component of microemulsion with 0-100% concentration in the increment of 10%. If four or more components are investigated for microemulsion system, binary mixtures like surfactant/co-surfactant or oil/drug are taken in the ordinates and pseudo ternary phase diagram will be constructed. (16)

The objectives of our present study were to formulate the microemulsion and mucoadhesive microemulsion for Nasal delivery for BCS Class-II drug, Vinpocetine, used for the management of disorder like ADHD and associated Dyslexia by enhancing memory. The formulations were optimized by using D-Optimal Mixture Design and was subjected for physicochemical characterization, *in vitro* and *ex vivo* evaluation.

5. 8 Materials and Instruments

Vinpocetine was kindly gifted as a Gift Sample by Micro lab ltd., Bangalore.

Table 5.25 List of Drug and Excipients

Sr. No.	Material	Name of Material	Manufacturer
1.	**Drug**	Vinpocetine	Micro lab ltd., Bangalore
2	**Oil**	Capmul MCM C8	Abitech Corporation, USA
		Labrafil 2125 Cs	Gattefosse, France
		Labrafac lipo	Gattefosse, France
		Soyabean oil	S.D. Fine Chemicals, Mumbai, India
		Miglyol-812	Abitec Corporation, USA
		Cottonseed oil	S.D. Fine Chemicals, Mumbai, India
		Captex-200	Abitec Corporation, USA
		Ethyle oleate	S.D. Fine Chemicals, Mumbai, India
3	**Surfactant and Co-surfactant**	Cremophor EL	BASF, Mumbai, India
		Imwitor-380	Gattefosse , France
		HCO_4O	Gattefosse, France
		Acconon CC6	Abitec Corporation, USA
		Tween 80	Spectrochem, Vadodara, India
		Labrasol	Gattefosse , France
		Solutol	Gattefosse , France
		PEG-400	Spectrochem, Vadodara, India
		Propylene Glycol	Spectrochem, vadodara, India
		Glycerin	SD Fine Chemical Lab. Mumbai
		Alcohol	SD Fine Chemical Lab. Mumbai
		Span 80	Spectrochem, Vadodara, India
5	**Mucoadhesive**	Chitosan	Sigma Aldrich

Equipments used are listed in Table 5.2 and purified HPLC grade water was obtained by the method described in section 5.2.

5.9 Preformulation Studies

Characterization of the Vinpocetine was performed in a same manner as discussed for Modafinil in 5.3.1.

5.9.1 Screening excipients

5.9.1.1 Screening of excipients based on the solubility study

For formulating microemulsion, preparation of Pseudo ternary phase diagram is an essential step to ascertain the quantity of oil, surfactant and co-surfactant but prior to that one must select appropriate excipients to form stable drug loaded microemulsion. (17) The most important criterion for the screening of components for microemulsion is the solubility of drug in oils, surfactants and co surfactants. (18)

5.9.1.2 Solubility Determination

The solubility study of Vinpocetine in different oil, surfactant and co-surfactant was performed as per the method discussed in section 5.3.2.1.

5.9.1.3 Screening of excipients based on the emulsification efficiency

The emulsifying ability was evaluated by measuring the % transmittance (% T) of the mixture primarily prepare with oil and surfactant/co-surfactant.

5.9.2 Drug-Excipients Compatibility Study using FT-IR

A preformulation study was carried out with formulation excipients to determine drug-excipients interaction/compatibility. Chemical compatibility can be determined by performing FT-IR in order to evaluate the interaction between drug and excipients. (19)

5.9.3 Screening of Surfactant: Co-surfactant (Smix) Ratio based on Pseudo Ternary Phase Diagram

Screening of excipients were based on method described on section 5.3.4. (18, 20)

5.10 Formulation of Microemulsion & Mucoadhesive Microemulsion

Formulation of microemulsion with mucoadhesive property require addition of the polymer. The method of preparation can be understood from Fig. 5.15. Based on the phase diagram, the optimum Smix ratio was selected and the drug loaded microemulsions were prepared by dissolving the drug in the oil, add Smix to it. The external phase was added in a drop wise manner under vortex mixing. (17) Considering the amount and solubility of drug to be incorporated in the microemulsion, certain microemulsions formulations were prepared

within the microemulsion region and the final composition of microemulsion was optimized based on transparency, dilution characteristics and globule size. Mucoadhesive formulations were prepared by adding chitosan to sodium acetate solution as aqueous phase.

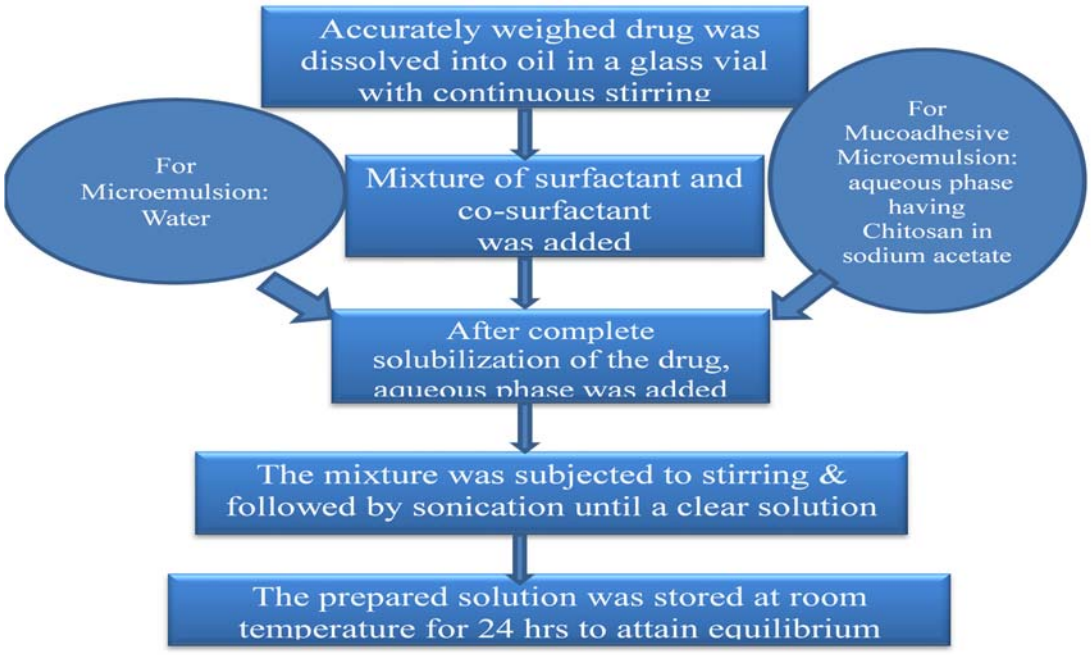


Fig. 5.15 Vinpocetine microemulsion and mucoadhesive microemulsion preparation

5.11 Optimization of Microemulsion by D-Optimal Mixture Design

This experimental design was used as a statistical tool to quantify relation between critical formulation variables and measured response for optimization. Based on the preliminary screening studies of microemulsion components A, B and C factors were identified as key factors responsible for the change in the of microemulsion shown in Table 5.26.

Independent variables A, B and C were taken in three levels and their minimum as well as maximum concentration was determined in the preliminary trial, from which design created by the software and it was characterized for specified parameters. Many formulations were prepared based on D-Optimal Mixture experimental design and were assessed for globule size (Y1) and % Transmittance (Y2) after 1:10 time dilution, their desired values were discussed in table 5.26.

Table 5.26 Variables with Desired Criteria of Dependable Variable

Sr. No.	Independent variables		
1	A	Amount of Oil	
2	B	Amount of S-Mix	
3	C	Amount of Aqueous Phase	
	Dependable variable		Criteria
1	Y1	% Transmittance	Maximum (100%)
2	Y2	Globule Size (nm)	Minimum (< 100 nm)

Optimized batch of the microemulsion system was assessed further for the optimization of the chitosan (mol. wt: 1,10,000-1,50,000) concentration for mucoadhesive microemulsion.

5.11.1 Optimization of Formulation

The formulation was optimized by D-optimal mixture design using Design Expert 9.0 software. The 3 factors to be optimized were: amount of oil, surfactant and co-surfactant. The runs were evaluated for the response variables and response values were subjected to multiple regression analysis to find out the relationship between the factors used and the response values obtained. (21)

5.11.2 Statistical Analysis

5.11.3 Optimization of Chitosan Concentration for Microemulsion System

Many excipients were acceptable for the preparation of the mucoadhesive microemulsion but chitosan provides added advantages like good solubility at acidic nasal pH, less prone to precipitation and enhanced drug transport across the nasal mucosa. Optimized microemulsion system was selected and optimum concentration of the chitosan was further assessed. Different concentrations of chitosan solutions were evaluated for the visual inspection on dilution, viscosity, globule size and % Transmittance.

5.12 Result and Discussion

5.12.1 Organoleptic Characteristics

Preliminary study of Vinpocetine show white, odorless and amorphous powder. Thus, visual identification of drug was confirmed that it did not show any sign of impurity.

5.12.2 Melting Point

Melting point of Vinpocetine was found to be in the range of 149-152°C which can be correlated with the standard reported value 147-153°C. The result shows that drug was in the pure form.

5.12.3 FT-IR study of Pure Drug

The characteristic absorption peaks of Vinpocetine were obtained from FT-IR study. The characteristic functional groups of the drug along with their wavenumbers in cm^{-1} are shown in Table 5.27. The FTIR spectrum of Vinpocetine is shown in Fig. 5.16.

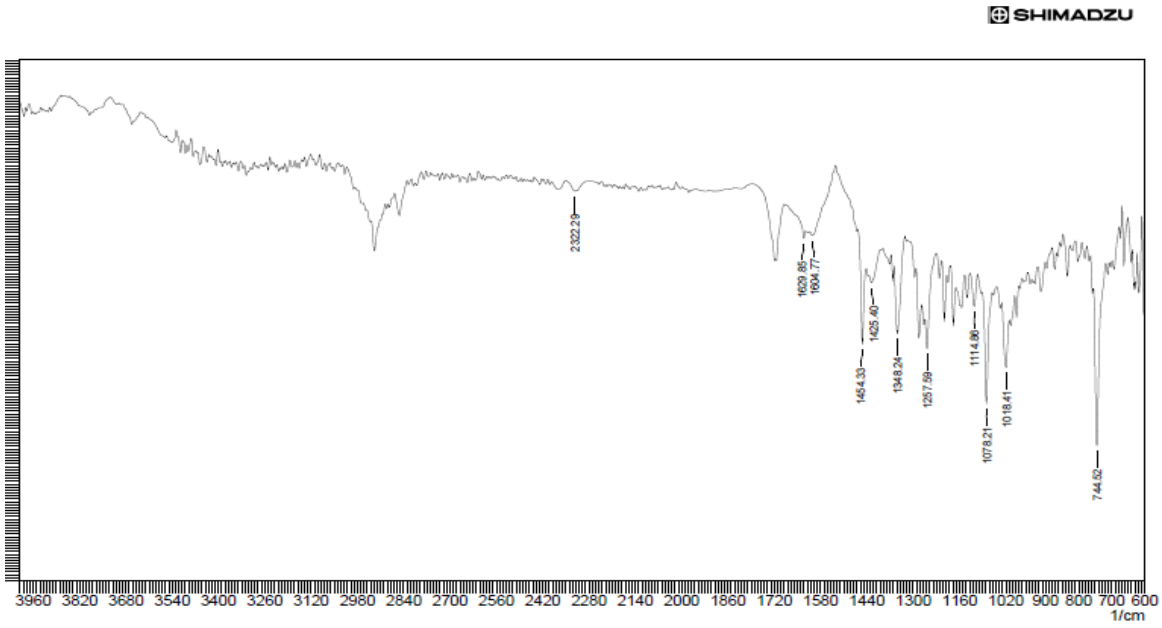


Fig. 5.16 FT-IR Spectra of Vinpocetine

From the results of FT-IR spectra of Vinpocetine, it was found that all characteristic peaks of function group were present in the drug. The result confirmed the drug was Vinpocetine without any traces of impurity.

Table 5.27 Characteristic Peaks of FT-IR Spectra of Vinpocetine

Sr. No.	Functional Group	Observed Wavenumber	Reported Wavenumber
1	-CH Stretching	2900 cm^{-1}	2850-3000 cm^{-1}
2	-C=O	1720 cm^{-1}	1730-1715 cm^{-1} aromatic ester
3	-C=C Stretching of aromatic ring	1630 cm^{-1} and 1605 cm^{-1}	Two peaks in 1630-1590 cm^{-1}
4	-C-H bending of methylene $–CH_2-$	1454 cm^{-1}	1450-1480 cm^{-1}
5	-C-O-C stretching of ester	1258 cm^{-1}	1290-1180 cm^{-1}
6	-C-O stretching	1115 cm^{-1} and 1078 cm^{-1}	1050-1150 cm^{-1}
7	-C-H bending of 1:2-substituted benzene	744 cm^{-1}	760-740 cm^{-1}

5.12.4 Thermal Behaviour of Drug by DSC

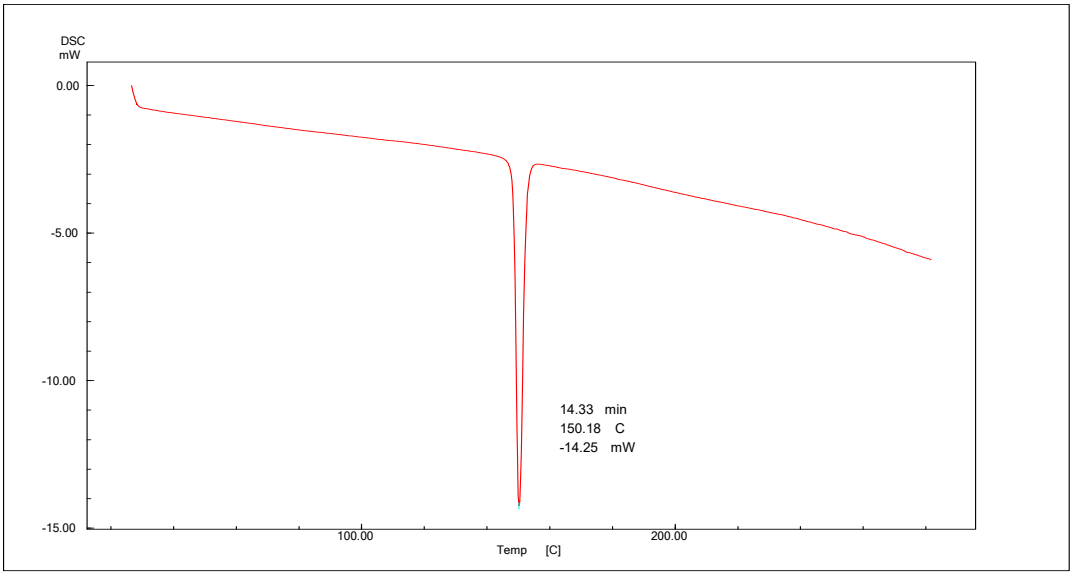


Fig. 5.17 DSC Thermogram of Drug: Vinpocetine

Fig. 5.17 represent DSC thermogram of drug showing sharp endothermic peak and no other degradation peaks, devoid of any solvent peak and moisture peak. This concludes that given

sample of drug was pure without any impurity. This finding was supported by the data obtained from capillary method and reported value represented in Table 5.28,.

Table 5.28 Melting Point of Drug using Different Methods

Melting Point		
Reported	**Capillary Method**	**DSC**
147-153°C	149-152°C	150.18 °C

5.12.5 Screening Excipients based on Solubility Study

Solubility of Vinpocetine in different oils, surfactants and co-surfactants is given in Fig. 5.18. Microemulsion must remain optically clear system even after dilution of microemulsion and for that, the components used in the system should have high solubilization capacity for the drug. The results are expressed in Table 5.29.

Table 5.29 Solubility of Vinpocetine in Different Oils, Surfactants and co-surfactants

Sr. No.	Excipients	Solubility (mg/ml)*	Sr. No.	Excipients	Solubility (mg/ml)*
1	Capmul MCM C8	22.02 ± 1.41	11	Acconon CC6	5.05 ± 1.19
2	Labrafil 2125 Cs	19.12 ± 0.66	12	Cremophore RH-40	17.34 ± 0.16
3	Labrafac lipo	18.54 ± 0.94	13	Imwitor-380	16.72 ± 0.57
4	soyabean oil	5.3 ± 1.52	14	HCO4O	2.22 ± 0.92
5	Miglyol-812	6.25 ± 1.73	15	Solutol	3.21 ± 0.67
6	Cottonseed oil	11.36 ± 0.81	16	Propylene glycol	18.12 ± 1.22
7	Captex-200	13.35 ± 1.49	17	PEG -400	22.63 ± 0.82
8	Ethyle oleate	15.89 ± 0.43	18	Span 80	3.14 ± 0.44
9	Labrasol	20.31 ± 1.30	19	Glycerin	6.05 ± 0.86
10	Tween-80	22.54 ± 0.68	20	Alcohol	14.35 ± 0.94

*mean ± S.D, (n=3)

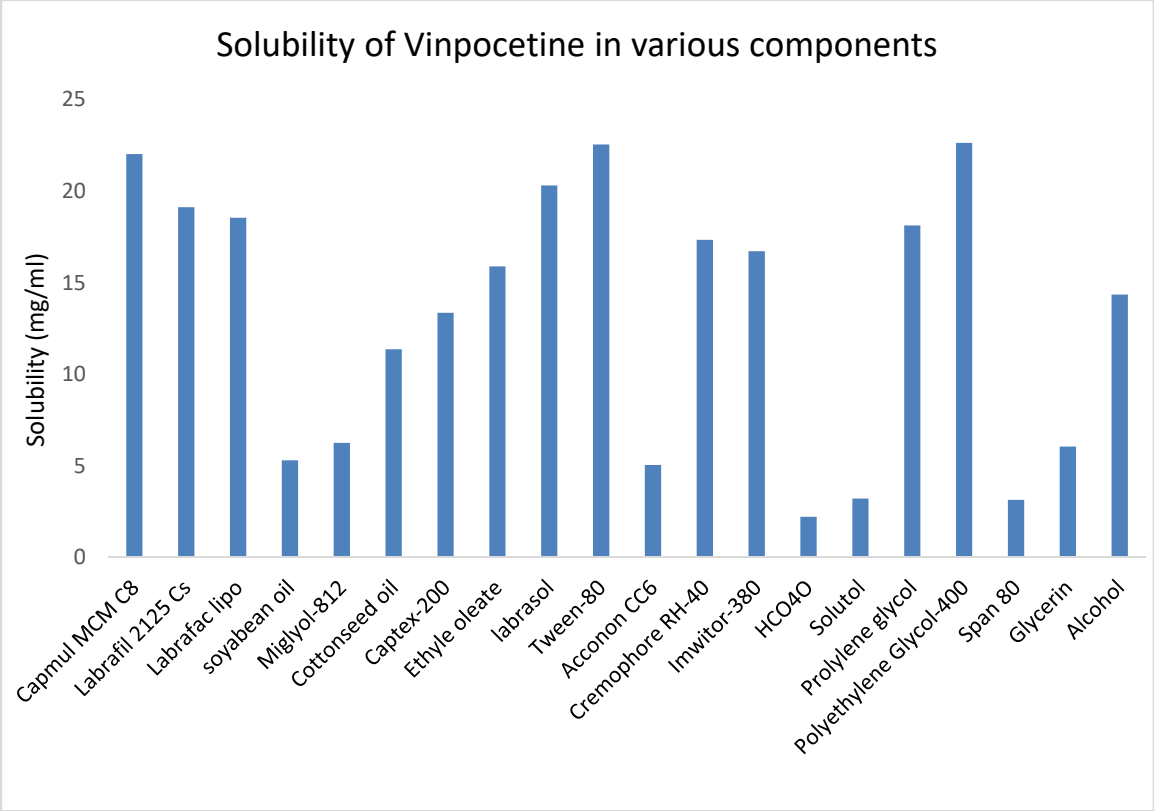


Fig. 5.18 Solubility of Vinpocetine in Different Oils, Surfactant and Co-surfactant

Maximum solubility of Vinpocetine was found to be in the Capmul MCM C8 (22.02 ± 1.41 mg/ml) followed by Labrafil 2125 Cs (19.12 ± 0.66 mg/ml) and Labrafac lipo (18.54 ± 0.94 mg/ml). If solubility of the drug is high in the oil, it may lead to lower concentration of surfactant and co-surfactant being used. Vinpocetine was found to have very low solubility in other oils than the Capmul MCM C8. Capmul MCM C8 is reported to have useful emulsifying properties for nasal delivery and it is classified in the GRAS (generally regarded as safe) component. Thus, the same was chosen as the oily carrier phase for the system.

The solubility of the drug was found maximum in surfactant Tween 80 (22.54 ± 0.68 mg/ml) followed by Labrasol (20.31 ± 1.30 mg/ml). The solubility of the drug was found maximum in co-surfactant Polyethylene Glycol (PEG)-400 (22.63 ± 0.82 mg/ml) followed by Propylene glycol (18.12 ± 1.22 mg/ml). For trial of formulating stable microemulsion, Tween 80 and PEG-400 was selected as a surfactant and co-surfactant respectively.

5.12.6 Screening of Excipients based on Emulsification Efficiency

From the solubility study, Capmul MCM C8 and Tween 80, PEG-400 were confirmed for formulation of the microemulsion as an oil surfactant and co-surfactant respectively. The selection of this combination was further evaluated based on the emulsifying abilities to emulsify the selected oil and was determined by measuring % transmittance of the prepared emulsion and are represented in Table 5.30. The values are expressed as mean±S.D. (n=3).

Table 5.30 Emulsification Efficiency of Surfactant and Co-surfactant

Sr. No.	Oil : Surfactant (1:20)	% Transmittance (at 650 nm)
1	Capmul MCM C8: Tween 80	99.45± 0.85
2	Capmul MCM C8: PEG-400	98.67 ± 0.93

From the results of emulsification efficiency, it can be concluded that Capmul MCM C8 oil showed highest transmittance with tween-80. Since, tween 80 was found to be giving transmittance 99.45%, it indicates its good emulsification ability and have shown considerable transmittance with PEG 400 with value 98.67%. This emphasizes the good result of globule size in the trial batches. Thus, it was selected for further study.

5.12.7 Drug Excipients Compatibility Studies

From the data of the solubility study, Capmul MCM C8, Tween-80 and PEG-400 were selected for the microemulsion. Compatibility of selected excipients with drug were analysed by IR spectra; IR spectra of drug (Fig. 5.16) compared with the IR spectra of drug loaded mixture of oil, surfactant and co-surfactant (fig. 5.19).

IR spectrum of Vinpocetine (Fig. 5.16) exhibits characteristic peak of the -CH stretching is in required range of 2850 cm^{-1}-3000 cm^{-1}. For keto group C=O stretch in aromatic ester is in required range of 1730 cm^{-1}-1715 cm^{-1}. Characteristic two peaks of -C=C stretching of aromatic ring is in required range of 1630 cm^{-1}-1590 cm^{-1}, -C-O-C stretching of ester is in required range of 1290 cm^{-1}-1180 cm^{-1}, -C-O stretching is in required range of 1115 cm^{-1} and 1078 cm^{-1}, -C-H bending of 1:2-substituted benzene is in required range of 760 cm^{-1} - 740 cm^{-1}. These peaks can be considered as characteristic peaks of Vinpocetine.

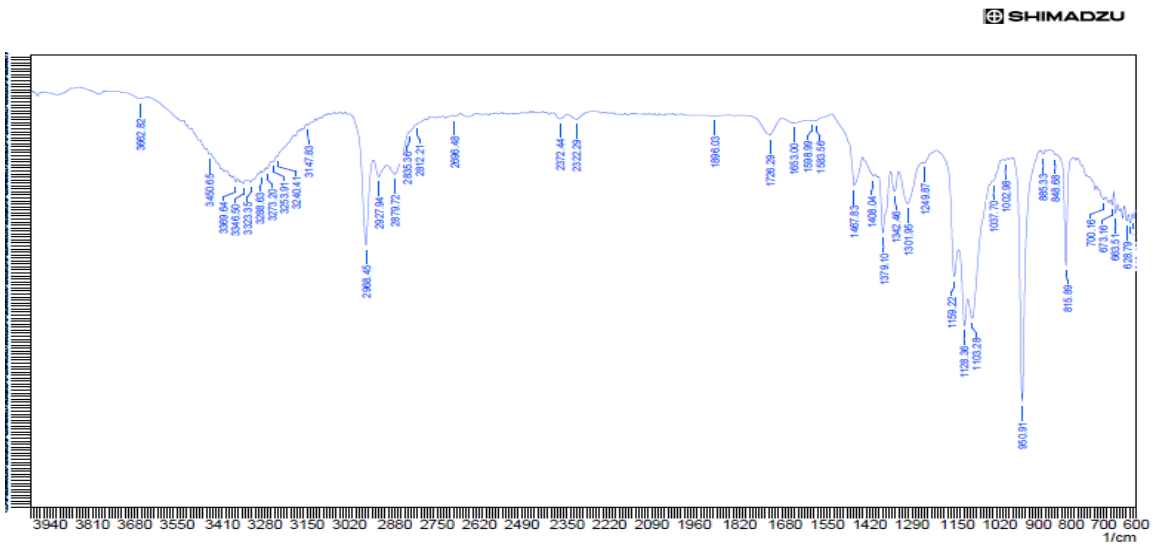


Fig. 5.19 FT-IR Spectra of Drug loaded Mixture of Capmul MCM C8, Tween 80 and PEG-400

IR spectra for Vinpocetine along with excipients was shown in the above Fig.5.19. Characteristic peaks of Vinpocetine were retained in IR spectra of Vinpocetine along with the mixture of oil (Capmul MCM C8), surfactant (Tween-80) and co-surfactant (PEG-400). Characteristic peaks of the individual excipients were also retained, also no new peak was found in drug loaded mixture of the excipients which is to be formulated in microemulsion. From these results, it can be concluded that there was no interaction between Vinpocetine and excipients of the formulation.

5.12.8 Screening of Surfactant: Co-surfactant (Smix) Ratio based on Pseudo Ternary Phase Diagram

Pseudo ternary phase diagrams were constructed to identify the microemulsifying region and ratio as well as type of surfactant and co-surfactant for formulating microemulsion which shows stability on infinite dilution with sodium acetate buffer pH 5.0 (SAB pH 5.0). Since surfactant and co-surfactant adsorb at interface and provide mechanical barrier to hinder coalescence, that's why Smix ratio [S/Co-S (km)], plays an important role in stable microemulsion formation.

Initially, the concentration of oil taken was maximum, i.e. 90%, and amount of S/Co-S was kept to minimum, i.e., 10%. Gradually, oil concentration was decreased and that of S/CoS

was increased. It was observed during these experiments that high concentration of oil forms poor emulsion with very low entrapment efficiency of SAB pH 5.0 and upon dilution with the same it shows hazy appearance. The pseudoternary phase diagrams were constructed at the ratios of S/Co-S or Tween 80/ PEG-400 (km) 3:1, 2:1 and 1:1 using Capmul MCM C8 as an oil and SAB pH 5.0 as an aqueous phase.

Phase diagram preparation shows that concentration of oil was found to be a rate-limiting factor for stable microemulsion preparation. At high concentration of oil, poor emulsion region was resulted. The black region shows stable microemulsion region. At any point beyond this boundary, microemulsion if formed initially, become turbid on further dilution of solution which indicates formation of emulsion with higher globule size (>200 nm).

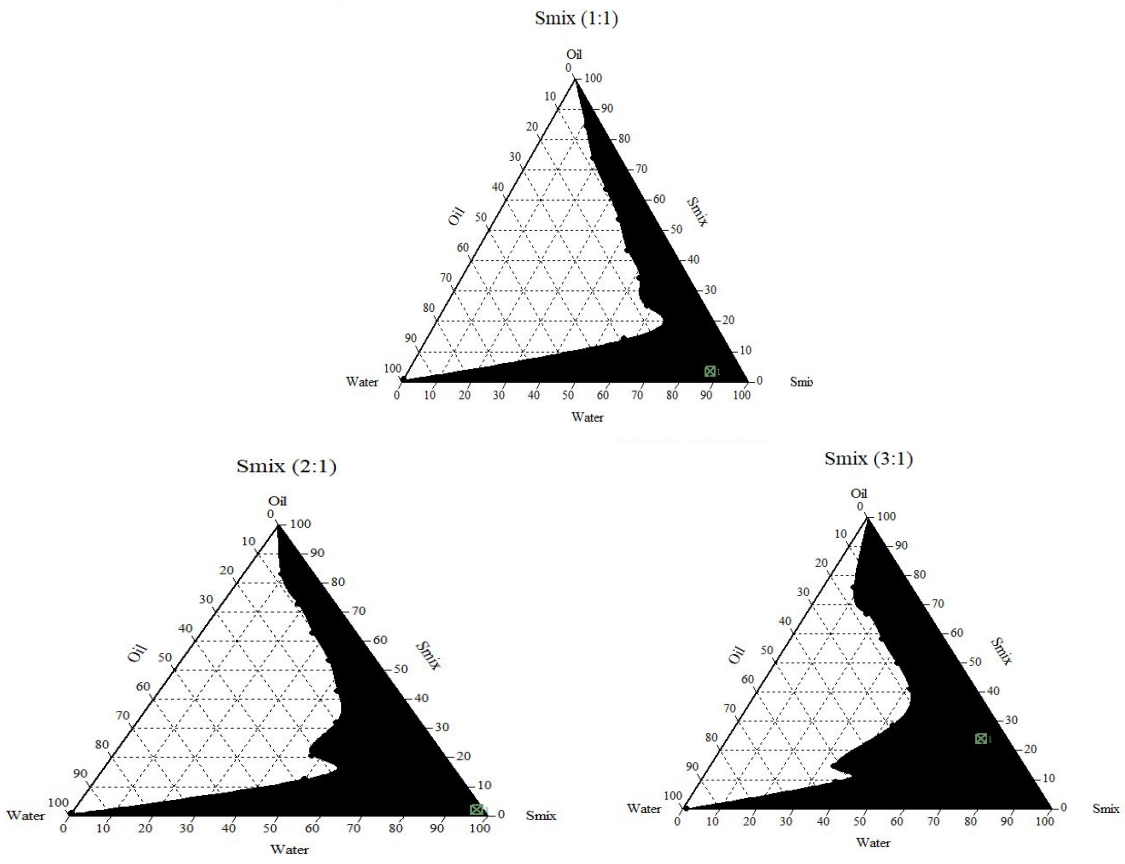


Fig. 5.20 Pseudoternary Phase Diagram at Different Km Value

The pseudo ternary phase diagram from Fig. 5.20 exhibits microemulsion region shaded with black colour. On construction of phase diagram and comparison of microemulsion

region obtained for all ratios of Smix, it was observed that, as the ratio changes from 3:1 to finally 1:1, it decreases black region of microemulsion formulation respectively. Thus, it was found that increased surfactant concentration results in good micro emulsion region for formulation. Here, Km ratio 3:1 and 2:1 shows stable microemulsion formulation. However, Km 3:1 had higher concentration of surfactant and leads to gastric irritation and other side effects. Here Km 3:1 shows lesser concentration of co-surfactant while drug has maximum solubility in the co-surfactant. So, to impart more drug loading, Km ration 2:1 was selected for further analysis.

5.12.9 Finalization of Concentration Range for the Microemulsion Formulation

Based on the results of the solubility studies, emulsification efficiency and preliminary study, Capmul MCM C8, Tween 80 and PEG-400 was selected as oil, surfactant and co-surfactant respectively. Table 5.31 depicts final composition for Vinpocetine microemulsion formulation.

Table 5.31 Concentration Range for the Optimization of Microemulsion (5ml batch)

Sr. No.	Class	Name of Excipients	Concentration Range
1	Drug	Vinpocetine	25 mg
2	Oil	Capmul MCM C8	4-8 %
3	Smix	Tween-80 and PEG-400	30-60 %
4	Aq. Phase	Water/ Sodium acetate buffer pH 5	30-70 %

5.12.10 Optimization of Microemulsion using D-Optimal Mixture Design

Applying a design offers comparative tests, screening, characterization, optimization and robustness of formulation. Statistical significance of these factors is established with analysis of variance (ANOVA) and graphical tools help identify the impact of each factor on the desired outcomes and reveal abnormalities in the data.

If formulation is a mixture, then D-Optimal Mixture Design is best suited and is most commonly used because they allow the most flexibility among the component ranges. Mixture design can have an irregular experimental region. The triangle in Fig.21 shows a design with the three factors A, B and C. Normally, all corners of the triangle are reachable, but in this example the factors are constrained. For mixture factors a constraint means that the lower and upper bounds differ from 0 and 1.

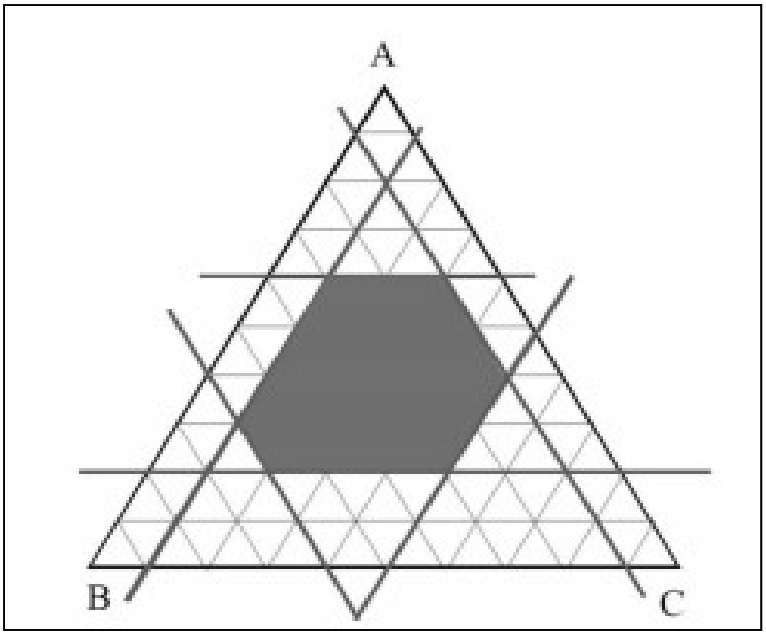


Fig. 5.21 Design with the Three Factors A, B and C

The reasons for using D-Optimal designs instead of standard classical designs are:

1. Standard factorial or fractional factorial designs require too many runs for the amount of resources or time allowed for the experiment.
2. The design space is constrained

Concentration of the excipients was selected based on (pseudo)ternary mixture designs, which is demonstrated in Table 5.32.

Table 5.32 Levels of Independent Factors in Experimental Design

Coded Values	ACTUAL VALUES		
	A : Oil (Capmul MCM C8)	**B: Smix** (Tween 80)	**C:Aqueous** (PEG-400)
Low Actual	4	30	34
High Actual	8	60	66

Table 5.33 D- Optimal Mixture Design for the Optimization of Microemulsion

Run	A: Oil (%)	B: Smix (%)	C: Aqueous (%)	Y1: Size (nm)	Y2:% Transmittance
1	5.984	60.000	34.052	32.57	98.6
2	8.000	46.065	45.935	40.19	97.7
3	8.000	38.793	53.207	312.52	15.8
4	4.002	34.778	61.219	74.29	97.6
5	4.000	30.006	65.994	198.2	55.5
6	8.000	55.293	36.707	26.01	98.7
7	8.000	55.293	36.707	27.40	98.8
8	4.000	38.498	57.502	23.75	99.2
9	4.000	57.665	38.335	16.56	98.4
10	5.756	43.637	50.607	105.16	98.8
11	5.984	60.000	34.052	27.56	98.1
12	4.000	30.006	65.994	193.5	56.2
13	5.201	50.601	44.198	14.95	99.2
14	4.000	57.665	38.335	17.13	99.0

A: Oil concentration (%w/w), B: Mixture of Surfactant and Co-surfactant concentration (%w/w), C: Aqueous concentration (%w/w), Y1: Globule Size (nm), Y2:% Transmittance.

5.12.11 ANOVA Analysis for Response Y1: Globule Size

Model selection for the globule size analysis was done by comparing F and P values for different models and whichever model show lesser F and P value was selected as the best suitable model. From Table 5.34 it confirmed that it follows Quadratic model. Lac of fit and model summary shows quadratic model as a best suitable model. The mean globule size was selected as a response and was represented in Table 5.33. It was ranged between 14.95 to 312.52nm, which indicate that the response was sensitive towards the studied factor. To identify the significant parameters and their interactions, analysis of variance was performed for each parameter. The values of the coefficients A, B and C are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response. Larger coefficient means the independent variable has more potent influence on the responses. Coefficients with P-value less than 0.05 had a significant effect on the measured response. The ANOVA for the mean globule size was represent in Table 5.35.

Table 5.34 Selection of the Model for Globule Size Analysis (Statistical Analysis)

Source	Sum of squares	df	Mean Square	F Value	p-value Prob>F	
Mean vs Total	63929.33	1	63929.3287			
Linear vs Mean	34565.21	2	17282.6064	3.052523	0.0882	
Quadratic vs Linear	34274.33	3	11424.7774	3.263656	0.0804	Suggested
Sp Cubic vs Quadratic	7734.152	1	7734.1521	2.670803	0.1462	Suggested
Cubic vs Sp Cubic	3143.603	3	1047.8676	0.244727	0.8615	
Residual	17127.10	4	4281.7759			
Total	160773.7	14	11483.8380			

Table 5.35 ANOVA Analysis of Experimental Design for Globule Size Analysis

Source	Sum of squares	df	Mean Square	F Value	p-value Prob> F	
Model	68839.55	5	13767.9090	3.933006	0.0426	Significant
Linear Mixture	34565.21	2	17282.6064	4.937031	0.0401	
AB	0.014283	1	0.0142	4.08E-06	0.9984	
AC	678.9515	1	678.9514	0.193952	0.6713	
BC	11388.3	1	11388.3035	3.253236	0.1089	
Residual	28004.86	8	3500.6073			
Lack of Fit	10877.76	4	2719.4387	0.635119	0.6646	Not significant
Pure Error	17127.1	4	4281.7759			
Core Total	96844.4	13				

The Model F-value of 3.93 implies the model is significant. There is only a 4.26% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case Linear Mixture Components are significant model terms. Values greater than 0.1 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve model. The "Lack of Fit F-value" of 0.64 implies the Lack of Fit is not significant relative to the pure error. There is a 66.46% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. Therefore this model can be utilized for further analysis. The following parameters shown in Table: 5.36 were deduced from ANOVA analysis:

Table 5.36 ANOVA Study Results for Globule Size Analysis

Sr. No.	Parameters	Results of Response (Y1)
1	Std. deviation	59.17
2	Mean	67.58
3	C.V.%	87.56
4	Press	1.061 E+005
5	R-squared	0.7108
6	Adjusted R-squared	0.5301
7	Predicted R-squared	-0.0954
8	Adeq Precision	6.937

A negative "Pred R-Squared" implies that the overall mean is a better predictor of response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 6.937 indicates an adequate signal. This model can be used to navigate the design space.

5.12.11.1 Mathematical Model for Globule Size (Y1)

Actual Equation

YI= -662.40*A +77.77*B +118.42*C -20.71*A*B +4516.03*A*C -455.67*B*C

From mathematical model for globule size, it was found that variables (A, B and C) and their interactions (A*B, A*C and B*C) had significant effect on Response 2. A shows negative effect suggesting an inversely proportional relationship with the size. Amount of oil was found to have negative effect, suggesting an indirect proportional relationship with the size. Also, its combination effect with aqueous phase shows positive impact. However, its combination effect with S-mix shows negative effect. It means that high concentration

of oil provides microemulsion with higher size in correlation with its combinatorial effect. S-mix and aqueous shows positive effect on size but their combination has negative effect on size.

The relationship between the dependent and independent variables was elucidated using 3D response surface plots in Fig. 5.21. The effect of A, B and C on globule size Y1 represent in Fig. 5.22, it reveals that the size of oil globules would be smaller at low value of oil and aqueous phase concentration. As the concentration of Smix increases globule size decreases.

5.12.11.2 3D Surface Plot

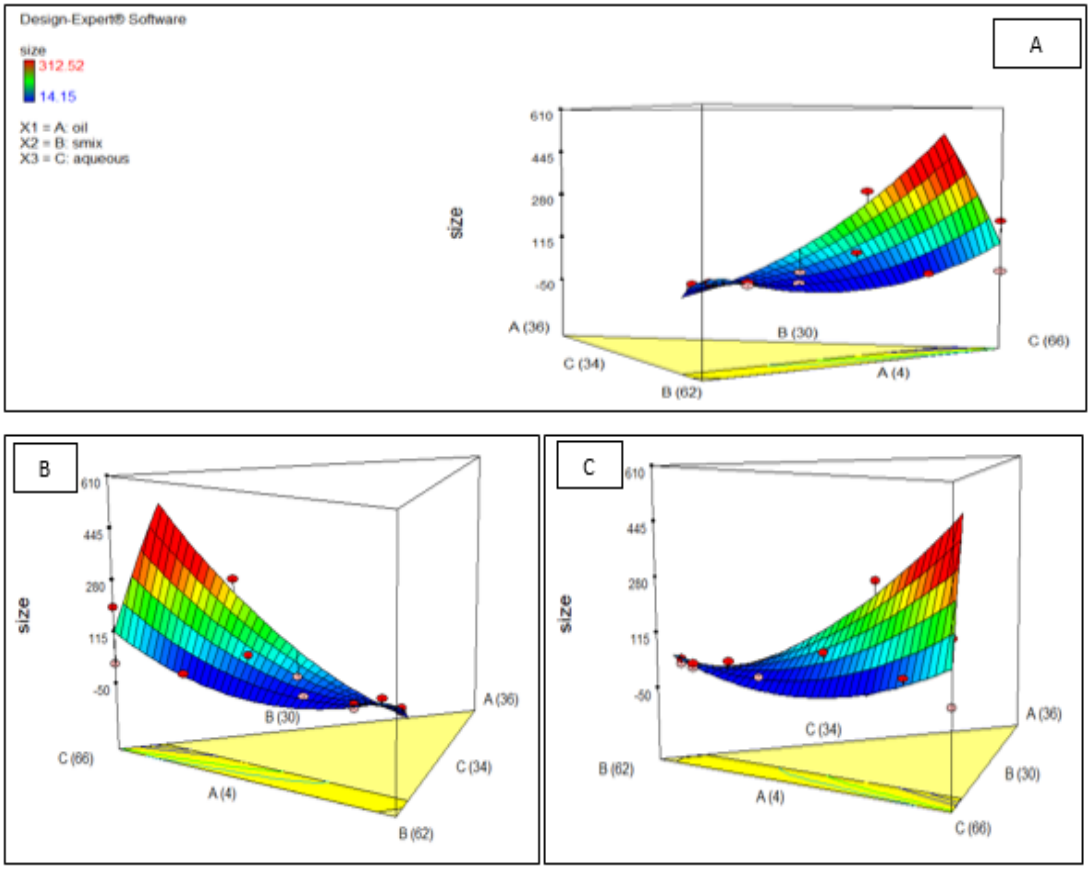


Fig. 5.22 Interaction effects of Oil, Aqueous phase and Smix on Globule size using 3D surface Plot: (A) as Oil decreases, size decreases, (B) as Aqueous phase decreases, size decreases, (C) as Smix increases, size decreases

5.12.12 ANOVA Analysis for Response Y2: % Transmittance

Model selection for the Transmittance analysis was done by comparing F and P values for different models and whichever model show lesser F and P value was selected as the best suitable model. It is represented in the Table 5.37.

Table 5.37 Selection of the Model for % Transmittance Analysis (Statistical Analysis)

Source	Sum of squares	df	Mean Square	F Value	p-value Prob>F	
Mean vs Total	112591.4464	1	112591.4464			Suggested
Linear vs Mean	2393.3463	2	1196.6731	2.5269	0.1250	
Quadratic vs Linear	3190.6706	3	1063.5568	4.2151	0.0460	Suggested
SpCubic vs Quadratic	739.2870	1	739.2870	4.0453	0.0842	
Cubic vs Sp Cubic	324.1094	3	108.0364	0.4524	0.7296	
Residual	955.15	4	238.7875			
Total	120194.01	14	8585.2864			

From Table 5.37, it has been confirmed that it follows Quadratic model. Lac of fit and model summary shows quadratic model as a best suitable model. % Transmittance was selected as a response and is presented in Table 5.33. It was ranged between 15.8% - 99.2%, which indicates that the response was sensitive towards the studied factor. The ANOVA for the % transmittance was represent in Table 5.38.

Table 5.38 ANOVA Analysis of Experimental Design for % Transmittance Analysis

Source	Sum of squares	df	Mean Square	F Value	p-value Prob>F	
Model	5584.0170	5	1116.8034	4.4261	0.0314	Significant
Linear Mixture	2393.3463	2	1196.6731	4.7427	0.0438	
AB	76.7435	1	76.7435	0.3041	0.5964	
AC	0.6852	1	0.6852	0.0027	0.9597	
BC	1498.4229	1	1498.4229	5.9386	0.0408	
Residual	2018.5465	8	252.3183			
Lack of Fit	1063.3965	4	265.8491	1.1133	0.4598	not significant
Pure Error	955.15	4	238.7875			

Cor Total	7602.5635	13				

The Model F-value of 4.426 implies the model is significant. There is only a 3.14% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case Linear Mixture Components, BC are significant model terms. Values greater than 0.1 indicate the model terms are not significant. The "Lack of Fit F-value" of 1.11 implies the Lack of Fit is not significant relative to the pure error. There is a 45.98% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. So this model can be utilized for further analysis. Therfore, this model can be utilized for further analysis.The following parameters shown in Table: 5.39 were deduced from ANOVA analysis:

Table 5.39 ANOVA Study Results for % Transmittance Analysis

Sr. No.	Parameters	Results of Response (Y1)
1	Std. deviation	15.88453081
2	Mean	89.67857143
3	C.V.%	17.71273845
4	Press	9317.789266
5	R-squared	0.734491329
6	Adjusted R-squared	0.568548409
7	Predicted R-squared	-0.22561149
8	Adeq Precision	7.488714396

A negative "Pred R-Squared" implies that the overall mean is a better predictor of response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.489 indicates an adequate signal. This model can be used to navigate the design space.

5.12.12.1 Mathematical Model for % Transmittance (Y2)

Actual Equation

Y2= -1009.23*A +77.39*B +74.94*C +1518.03*A*B +143.47*A*C +165.29*B*C

From mathematical model for globule size, it was found that variables (A, B and C) and their interactions (A*B, A*C and B*C) had significant effect on Response 1. A shows negative effect suggesting an inversely proportional relationship with the %Transmittance.

Amount of oil was found to have negative effect, suggesting an indirect proportional relationship with the %Transmittance but its combination effect with S-mix and aqueous phase shows positive impact. It means that high concentration of oil provides microemulsion with higher %Transmittance in correlation with its combinatorial effect. S-mix and aqueous shows positive effect on %Transmittance. % Transmittance of the emulsion is an important factor in SMEDDS formulation, as it determines stability of the formulation on *in vivo* application.

5.12.12.2 3D Surface Plot

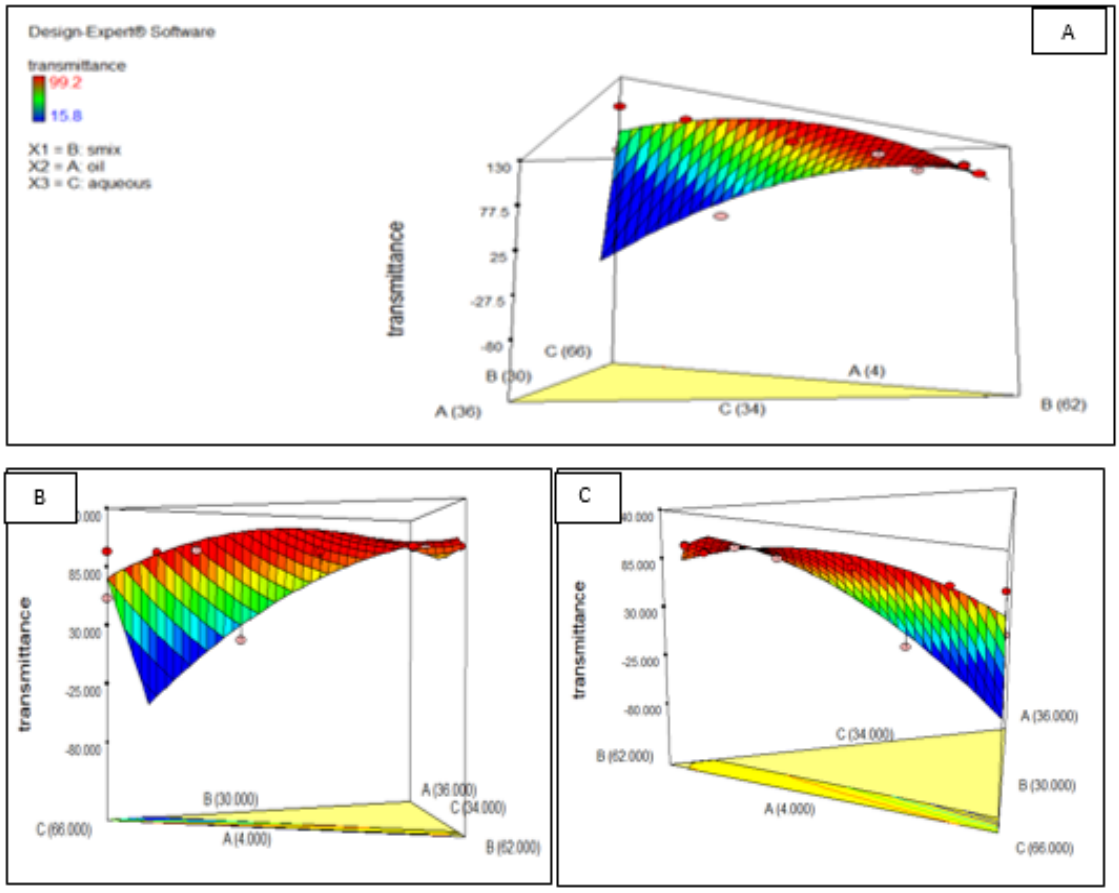


Fig. 5.23 Response Surface Curve (3D Plot) Showing Combined Effect of Independent Variables on % Transmittance. (A) as Oil Decreases, %T Increases, (B) as Aqueous Decreases, %T Increases, (C) as Smix increases, %T Increases

5.12.13 Numerical Optimization

As discuss earlier in Modafinil; Numerical optimization i.e. Desirability study for the optimization of microemulsion was obtained by lower and upper limit of Oil, Smix, aqueous

phase and with desired criteria limit of globule size and transmittance (Table 5.40). Table 5.41 and Fig. 5.24 represents the desirability batch generated by software with desirability near to maximum one; this batch was prepared, evaluated by experimentally for confirmation and optimization of microemulsion, result was tabulated in Table 5.42.

Table 5.40 Constraints Applied for Selection of Optimized Batch

Name	Goal	Lower Limit (%w/w)	Upper Limit (%w/w)
A: Oil	In range	4.0	8.0
B: S-Mix	In range	30.0	60.0
C: Aqueous	In range	34.0	65.0
% Transmittance	Maximize	98.0	100.0
Size (nm)	Minimize	20.0	80.0

Table 5.41 Formulation Parameters Based on Desirability

Oil	S-Mix	Aqueous	%Transmittance	Size(nm)	Desirability
4.000	56.6483	39.3517	100.0	19.1072	0.982

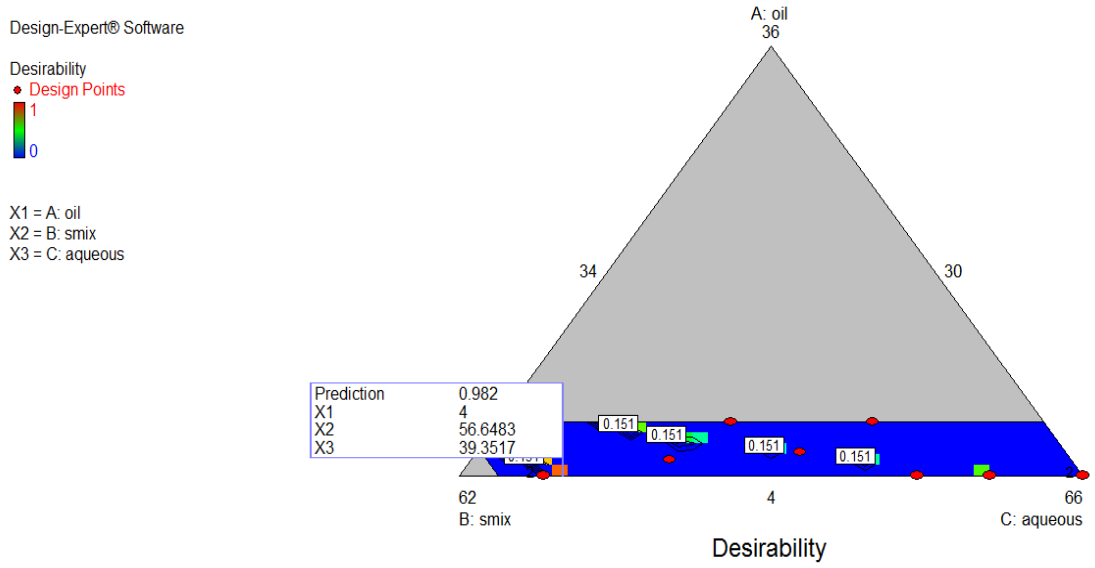


Fig. 5.24 Desirability Plot for Selection of Parameters

Table 5.42 Suitability of Predicted Desirability Plot for Optimized Formulation

Independent Variables	Predicted Values	
Oil	4.000	
S- Mix	56.6483	
Aqueous Phase	39.3517	
Response	**Predicted**	**Actual**
% Transmittance	99.9941	99.73 ± 0.658
Size (nm)	21.1215	19.01 ± 1.111

The Table 5.42 shows predicted response for the desired batch along with the standard deviation and 95% confidence interval of the response. The optimum composition was selected on the basis of desirability plot shown in Fig. 5.24. The factor A, B and C provided optimum response respectively at 4%w/w, 56.6483%w/w and 39.3517%w/w with 19.01 ± 1.111 nm of globule size and 99.73 ± 0.658% transmittance with desirability 0.982. T- Test values for both factors were lower than their T- standard values. Therefore, there was no significant difference ($p>0.05$) between predicted and experimental values of both the factors. Therefore, the model was validated. The result obtained by graphical representation

5.12.14 Graphical Optimization

Graphical Optimization by Overlay Plot for predicted design space; experimental design was used for multiple responses Y1: Globule size and Y2: % Transmittance, it is necessary to obtain a region that provides optimum values of factors. Overlay plot (Fig. 5.25) can be obtained by superimposing contour plots of responses Y1 and Y2 which displays the area of feasible response values in the factor space. The region highlighted in yellow color is the area in which a slight variation in the critical variables will not affect the final response and the response will be in desired range. Regions that do not fit the optimization criteria are shaded gray while design space, which is accepted, are colored yellow. Flag place in overlay plot show predicted values of desired response with optimized values of variables and are same observed in desirability. Table 5.42 represent that there is no significant difference between predicted and experimental value. Confirmation of the response was done by carrying out experiment using the selected factor values in triplicate. T- Test values for both factors were lower than their T- standard values at 95% confidence interval of the response.

This confirm that there was no significant difference between predicted and experimental values of both the factors. So, the model was validated.

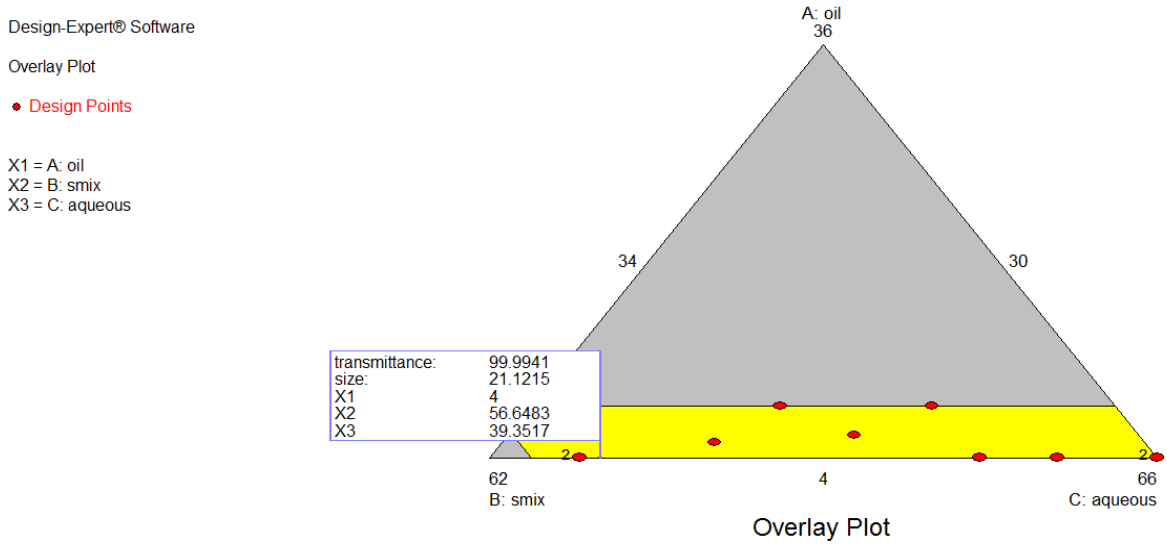


Fig. 5.25 Overlay Plot for Microemulsion

5.12.15 Optimized Batch of Vinpocetine Microemulsion

Table 5.43 Concentration of the Ingredients for Optimized Batch

Sr. No.	Component	Optimized Batch
1	Drug: Vinpocetine	25 mg/5mg
2	Oil: Capmul MCM C8	4.0 %
3	Smix (2:1) : Tween-80: PEG-400	56.65 %
4	Aqueous Phase: Sodium Acetate Buffer pH 5.0	39.35 %

5.12.16 Maximum Drug Loading in Optimized Batch of Microemulsion

Formulate different batches for the microemulsion and add specified quantity of drug in the increasing order. After each addition analyse preparation for globule size, % Transmittance at 0/4 hr and Stability on dilution at 0/4 hr.

Table 5.44 Effect of Drug Loading on Stability of the Microemulsion System

Sr. No.	Drug Loading (mg)/ 5 gm	Globule Size (nm)	Percentage Transmittance		Stability (Dilution 100 times)	
			0 hr	4 hr	0 hr	After 4 hr
F1	25	19.01±1.11	99.73±0.65	99.46±0.89	Stable	Stable
F2	30	20.14±1.03	99.95±1.46	99.54±1.44	Stable	Stable
F3	35	21.21±1.41	99.89±0.94	99.48±0.02	Stable	Stable
F4	40	21.43±1.87	99.99±0.78	99.89±1.40	Stable	Stable
F5	45	25.88±1.64	98.98±1.73	92.54±1.27	Stable	Precipitation

From table 5.44 it can be understood that drug loading doesn't affect globule size as a main responsive factor. Effect of drug loading evaluated for dilution stability which shows up to 40 mg of drug, it doesn't revel on dilution stability but on the addition of 45 mg of Vinpocetine it will shows instability on dilution within the time duration of 4 hr. So, F4 batch with drug loading efficiency of 40 mg can also be used. 25 mg/5mg of dose is sufficient for the further study as minimum dose.

5.12.17 Optimization of Chitosan Concentration for Mucoadhesive Microemulsion

Aqueous phase was sodium acetate buffer pH 5.0 at which chitosan shows good solubility. Chitosan has dual advantage that it can act as a mucoadhesive agent as well as permeation enhancer. Different batches were prepared to optimize its concentration.

Table 5.45 Optimization of Chitosan Concentration

Sr. No.	Percentage of Chitosan	Globule Size (nm)	Percentage Transmittance	Stability on Dilution (100 times)		
			0 hr	0 hr	After 4 hrs	After 10 hrs
M1	0.0	19.01 ± 1.11	99.73 ± 0.75	Stable	Stable	Stable
M2	0.1	22.11 ± 1.45	99.43 ± 0.43	Stable	Stable	Stable
M3	0.2	22.43 ± 0.88	99.44 ± 0.46	Stable	Stable	Stable
M4	0.3	21.82 ± 1.08	98.76 ± 0.41	Stable	Stable	Stable
M5	0.4	21.17 ± 1.16	98.44 ± 0.70	Stable	Stable	Stable
M6	**0.5**	**22.19 ± 1.28**	**98.03 ± 0.61**	**Stable**	**Stable**	**Stable**
M7	0.6	40.42 ± 1.76	85.16 ± 1.63	Translucent	Haze	-

Table 5.45 represents that stable microemulsion system must not be formulated with more than 0.6 % chitosan. As the concentration of chitosan increases, globule size was also increasing it show instability of the system. Batch M6 was selected as a finalized batch for the further characterization of the formulation as it remains stable at least for 10 hr.

5.12.18 Optimized Formula for Microemulsion and Mucoadhesive Microemulsion System

Table 5.46 represents formula for the optimization batch of ME and MME.

Table 5.46 Optimized formula for microemulsion and mucoadhesive microemulsion

Sr. No.	Ingredient	ME	MME
1	Drug	25 mg/5mg	25 mg/5mg
2	Capmul MCM C8	4 %	4 %
3	Smix (Tween-80 and PEG-400) 2:1	57.58 % (38.386 : 19.193)	56.65 % (37.77 : 18.88)
4	Double distilled water (ME) / Sodium acetate buffer pH 5.0 (MME)	37.416 %	39.35 %
5	Chitosan	-----	0.5 %

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