Chapter 7 (Part- A)

In Vitro & *Ex Vivo* Drug Release Study for Oral SMEDDS

Management of Dyslexia and ADHD

7.1 Introduction

7.1.1 *In Vitro* Drug Release

Without exception, *in vitro* release testing is an important analytical tool used to investigate and establish product behavior during the various stages of drug product development, as well as life cycle management. (1) An *in vitro* release profile can reveal fundamental information on the release mechanism and kinetics, enabling a rational and scientific approach to drug product development. (2) Despite the great strides in design and development of nano sized dosage forms, no compendia or regulatory standards exist for *in vitro* release testing. Although there have been attempts to use the existing USP apparatus for *in vitro* drug assessment of microemulsion. This information helps a prognostic approach to design and development of drug delivery system with required properties. *In* comparison to *in vivo* drug release studies, *in vitro* drug release studies have gained increasing importance because the *in vivo* studies include cost of labor and sacrifice of living beings. *In vitro* study used as a prognostic approach for *in vivo* behavior. (2) It is also used to check the batch variability of similar formulation by comparing drug release pattern.

There are two approaches to study drug release pattern from the SMEDDS formulation. First and permissive approach is the usage of type II dissolution apparatus and second is the release study using dialysis sac. Now, it has been understood that, when the SMEDDS encounter the aqueous medium, different forms of drug are formed: drug in the free molecular state, in microemulsion and in micellar. As conventional release, method is not appropriate for this system and to solve this problem dialysis bag/sac was used. (3) Artificial membranes have the advantage of offering highly reproducible system but it needs to be activated prior to its usage. In this investigation, the *in vitro* drug release from SMEDDS formulations of Modafinil was performed by both conventional dissolution method as well as dialysis bag method.

7.1.2 *Ex Vivo* Drug Permeability Study

It was performed on the isolated organ or tissue, under the identical environmental condition of body. *Ex vivo* absorption study or one can use more precise terminology "permeation study" of the formulation is the valuable tool to envisage behavior of

particular formulation with respect to drug transport across the stomach and/or intestinal membrane *in vivo*. (3, 4)

7.2 Materials and Instrumentation

Table 7.1 List of Material and Reagents

Chemical/Reagents	Manufacturer / Supplier
Potassium chloride, AR grade	S.D. Fine Chemicals, Mumbai, India
Sodium chloride, AR grade	S.D. Fine Chemicals, Mumbai, India
Sodium hydroxide, AR grade	Spectrochem Labs Ltd, Vadodara, India
Potassium dihydrogen phosphate, AR	Spectrochem Labs Ltd, Vadodara, India
Sodium dihydrogen phosphate, AR	Spectrochem Labs Ltd, Vadodara, India
Potassium dihydrogen orthophosphate	S.D. Fine Chemicals, Mumbai, India
Sodium Lauryl Sulphate	Spectrochem Labs Ltd, Vadodara, India
Concentrated Hydrochloric acid	Rankem chemical, Vadodara, India
Dialysis Membrane	HimediaPvt. Ltd, Mumbai, India
Distilled water	Prepared in the laboratory

Table 7.2 List of Equipments

Equipments	Manufacturer / Supplier
Digital Analytical balance	Shimdzu SCS, Switzerland
Dissolution Apparatus	USPXXIII apparatus I, Veevo ,Mumbai
Magnetic stirrer	Remi equipments Pvt. Ltd, India
HPLC-UV detector	Shimadzu, Japan

As per the Indian Pharmacopoeia 2007, solutions used for the study were prepared.

7.3 *In Vitro* Dissolution Study

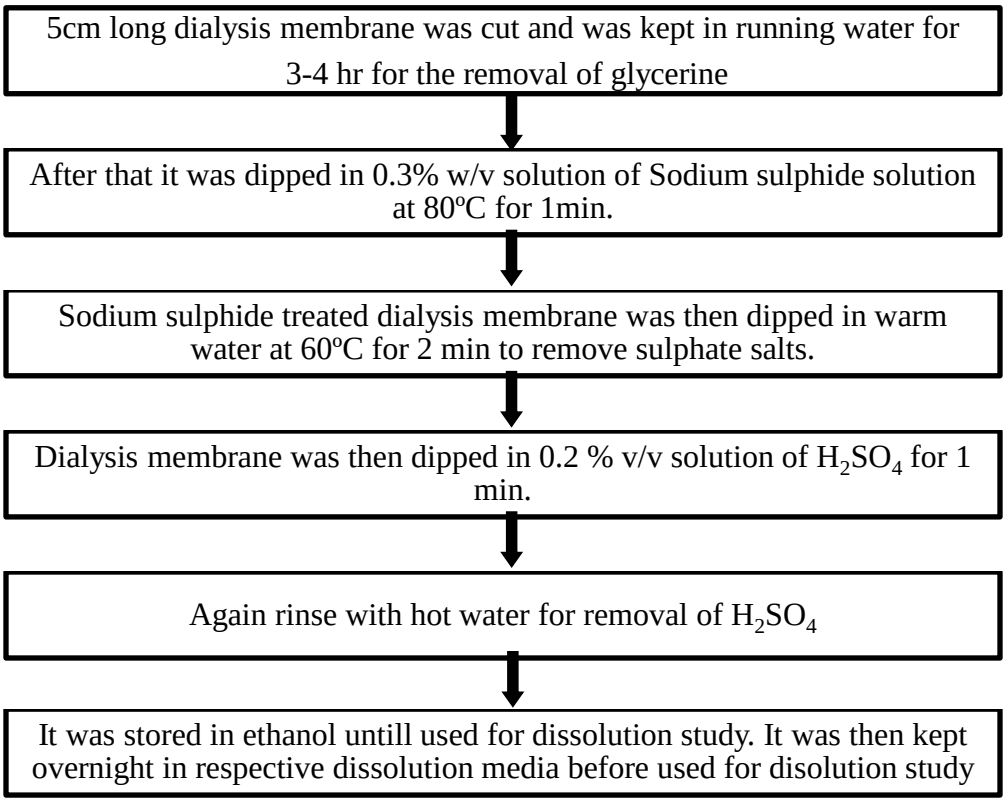
According to United State Pharmacopoeia (USP 30) dissolution procedure, USP type-II dissolution apparatus was used to performed dissolution test for SMEDDS (equivalent to 20 mg modafinil) and drug (20 mg) separately. Apparatus was loaded with 900 ml 0.1N HCL containing 0.5% sodium lauryl sulphate (SLS) at 37 ± 0.5^{o}C with paddle speed of 50 rpm. Each sample (5 ml) was withdrawn at predetermined interval of time and replaced sample by an equal volume of dissolution medium to maintain sink condition. Amount of dissolved modafinil was determined by RP-HPLC. Samples were filtered through 0.22 µm syringe filter, the resulting filtrate was suitably diluted with mobile phase and 20 µl was injected into the HPLC for analysis. Dissolution tests were performed in triplicate. (3) To

find out the effect of pH on dissolution profile of SMEEDS, the same procedure was followed for SMEDDS in phosphate buffer pH 6.8 with 0.5%SLS instead of 0.1 N HCl containing 0.5% and were performed in triplicate.

7.4 *In Vitro* Diffusion Study

7.4.1 Activation of Dialysis Membrane

Dialysis membrane 70 (LA393), having molecular weight cut off of 12000-14000 Da, average flat width of 28.46 mm, average diameter of 17.5 mm and approximate capacity of 2.41 ml/cm, obtained from Himedia Laboratories, was used for the study. Before using the dialysis membrane for drug release study it was first activated by following steps:(5)



7.4.2 *In Vitro* Diffusion Study Through Dialysis Bag

Dialysis sac was thoroughly washed with water, then it was filled with 1% SLS and examined for leaks and again washed. *In vitro* diffusion profile was studied by diluting1ml of SMEDDS (5mg Modafinil) up to 5ml with 0.1N HCl solution containing 0.5 % SLS and from this 1 ml of diluted SMEDDS (1 ml;5mg/ml) was filled into the activated dialysis sac. Finally, it was tied at the both ends with a thread and care was taken to ensure that there

was no leakage of the content from the prepared sac. Drug suspension was prepared in the same way by dispersing 5 mg of drug. The sac was mounted in glass beaker containing 1000 ml of phosphate buffer pH 6.8 (0.5% SLS). Here the sac was acting as a reservoir compartment and the respective media as the receptor compartment. The content of the beaker was stirred using teflon coated magnetic bead; beaker was covered with paraffin film to prevent evaporative loss of solution during the experimental run. (3, 6) At predetermined interval of time, specified quantity of sample (5 ml) was collected from receptor compartment and replaced with the same volume of diffusion media. Amount of the drug release was determined using RP-HPLC method. Same procedure was followed for drug suspension. All the studies were performed in triplicate and data are shown in Table 7.11 and graphically represented in Fig. 7.8. The % release of drug was calculated against time and plotted graph.

7.5 *Ex Vivo* Drug Permeability Study using Isolated Tissue of Rat

Male Rats (Sprague Dawley) (250-300 g) were sacrificed; the tissues of stomach and duodenum were isolated carefully, were cleaned properly with cold Ringer's solution to remove the mucous and lumen contents. Formulation was diluted with same media used for the receptor compartment. 1ml of diluted SMEDDS were instilled in to isolated stomach tissue, tied at both ends to avoid leakage of the formulation. (3, 4) Receptor compartment was filled by 0.5% SLS in 0.1N HCL with continuous aeration and tissue was suspended in the compartment at 37°C. At predetermined time intervals, samples were withdrawn from the receptor compartment and at the same time fresh media was replenished. The samples were analyzed by RP-HPLC. Repeated the same procedure for the suspension and repeated the whole experiment using duodenum for both SMEDDS and suspension in phosphate buffer pH 7.4 containing 0.5% SLS. Here magnetic bead rotated at 200 rpm and bath temperature was maintained at 37±0.5°C with continuous aeration throughout the experiment. The % cumulative drug release was calculated against time and the graph was plotted; experiment was performed in triplicate and data are shown in Table 7.11 and graphically represented in Fig. 7.8.

7.6 Result and Discussion

7.6.1 *In Vitro* Dissolution using USP Type II Apparatus

Table 7.3 *In Vitro* Dissolution Profile of Modafinil loaded SMEDDS, Pure Drug and Marketed Formulation (in 0.1N HCL with 0.5 % SLS)

Sr. No.	Time (hrs)	% Cumulative Drug Release ± SD (n=3)		
		SMEDDS	Pure Drug	Marketed
1	0.1	81.027 ± 5.689	11.387 ± 1.842	45.02 ± 4.212
2	0.25	93.274 ± 3.207	16.616 ± 2.520	60.55 ± 2.451
3	0.50	98.187 ± 0.718	19.259 ± 3.482	76.45 ± 0.897
4	0.75	98.991 ± 0.297	24.965 ± 2.685	88.71 ± 0.445
5	1.00	99.243 ± 0.028	31.328 ± 2.530	95.51 ± 0.041
6	1.50	99.299 ± 0.121	39.748 ± 1.607	98.81 ±0.032
7	2.00	99.329 ± 0.081	42.451 ± 0.689	-
8	3.00	99.416 ± 0.095	48.011 ± 1.146	-
9	4.00	-	53.917 ± 0.394	-
10	6.00	-	61.198 ± 0.917	-
11	8.00	-	63.192 ± 0.059	-

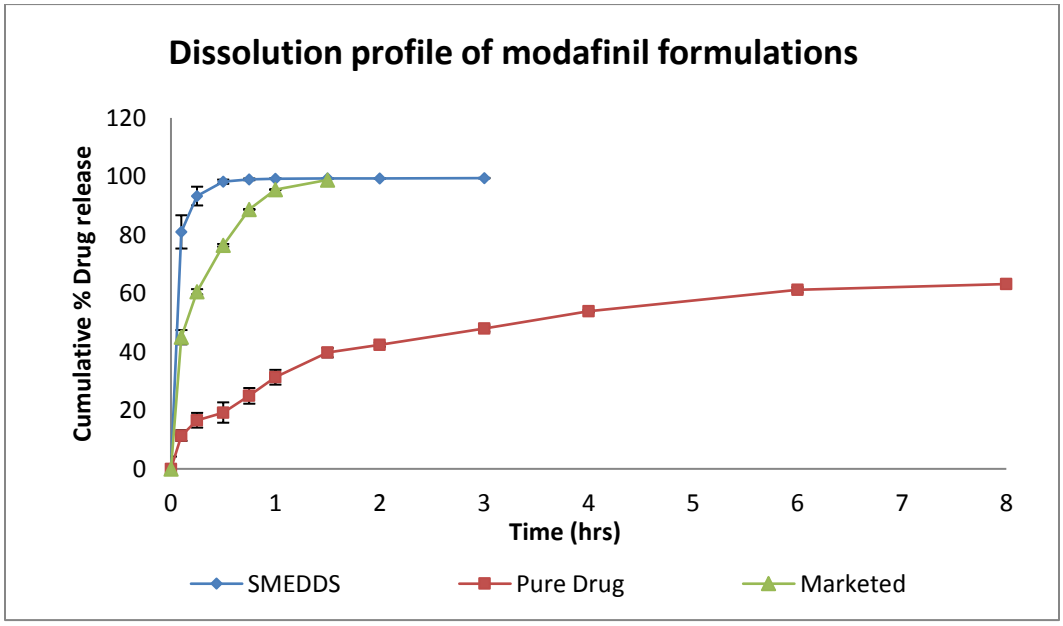


Fig. 7.1 Dissolution Profiles of SMEDDS, Drug and Marketed Formulation

Table 7.4 Effect of pH on Dissolution Profile of SMEDDS

Sr. No.	Time (min)	% Cumulative Drug Release ± SD (n=3) from SMEDDS	
		0.1 N HCL with 0.5 % SLS	Phosphate buffer pH 6.8 with 0.5 % SLS
1	6	81.027 ± 5.689	78.159 ± 3.424
2	15	93.274 ± 3.207	94.765 ± 2.147
3	30	98.187 ± 0.718	98.487 ± 0.142
4	45	98.991 ± 0.297	98.895 ± 0.358
5	60	99.243 ± 0.028	98.965 ± 0.017
6	90	99.318 ± 0.121	99.421 ± 0.024
7	120	99.329 ± 0.081	99.411 ± 0.026
8	180	99.326 ± 0.095	99.461 ± 0.034

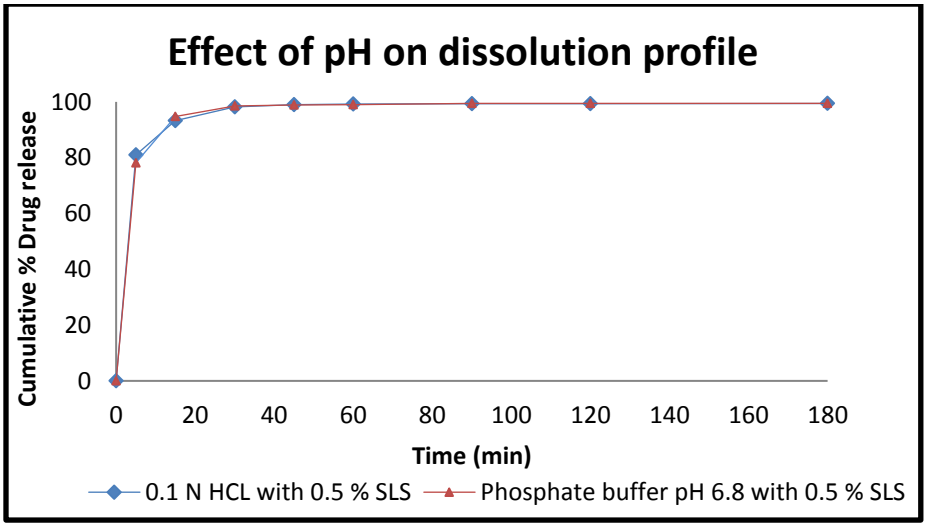


Fig. 7.2 Effect of pH on *In Vitro* Dissolution Profiles of SMEDDS

As shown in Fig. 7.1, Table 7.3, in vitro dissolution study revealed that drug released from SMEDDS (81.027 ± 5.689 %) was more than 80% in less than 10 min, which is approximately 7 times higher compared to pure drug (11.387 ± 1.842 %) and 1.8 times higher than the marketed formulation (45.02± 4.212). After 15 min., drug released from SMEDDS was 93.274 ± 3.207 % and from pure drug 16.616 ± 2.520% and from marketed was 60.55 ± 2.451%. SMEDDS shows almost complete drug release within 30 min whereas only 50% drug released from pure drug after 4 hours, while more than 90 min required to complete release of drug from marketed tablet. SMEDDS shows significant ($p<0.5$) improved dissolution profile compared to pure drug and marketed formulation.

This performance of drug release from SMEDDS was due to the nano size (<20 nm) of drug globule and size is inversely proportional to the surface area which is directly proportional to the dissolution. SMEDDS had smaller globule size and this system dealt with already dissolved form of the drug which facilitates improved drug release profile as compared to pure drug and marketed formulation. Moreover, solubilized form of drug in oil and Smix confirms that the solubility of the drug increases several times. Dissolution of Modafinil SMEDDS at different pH represent in table 7.4 and Fig. 7.2. Different pH media doesn't affect drug dissolution profile of SMEDDS, it shows that it provide pH independent drug release.

7.6.2 *In Vitro* Drug Diffusion Study by Dialysis Sac

Table 7.5 *In Vitro* Drug Diffusion Profile for SMEDDS and Suspension of Modafinil

Sr. No.	Time (hr)	Cumulative % Drug Diffusion ± SD (n=3)	
		SMEDDS	Drug suspension
1	0.1	6.989 ± 1.242	-
2	0.25	9.595 ± 0.821	-
3	0.5	12.153 ± 1.165	5.971 ± 0.465
4	0.75	15.669 ± 2.196	8.502 ± 0.968
5	1	20.096 ± 1.348	13.658 ± 0.852
6	1.5	33.768 ± 2.874	18.539 ± 0.079
7	2	40.169 ± 2.658	23.419 ± 1.215
8	2.5	43.652 ± 1.215	32.066 ± 1.686
9	3	52.971 ± 3.268	35.126 ± 0.865
10	4	68.101 ± 1.685	43.912 ± 0.961
11	6	82.369 ± .069	51.401 ± 1.359
12	8	87.633 ± 1.086	59.258 ± 0.236
13	24	99.308 ± 1.203	78.328 ± 0.0698

In vitro drug diffusion profile for modafinil SMEDDS and drug suspension are represented in Fig. 7.3. and Table 7.5, it revealed that modafinil diffused from suspension was very less as compared to SMEDDS. Approximately 50 % drug released from SMEDDS and from drug suspension; within 3 hrs and 6 hrs respectively. After 8 hrs drug release from SMEDDS was 87.633 ± 1.086 %, which was 1.5 times higher compared to 59.258 ± 0.236% of drug release from suspension. SMEDDS shows that drug release was improved and sustained compared to drug suspension. It may be because small globule size of

SMEDDS formulation provides an additional support for faster solubilization and diffusion from dialysis sac that result in faster release of drug than suspension.

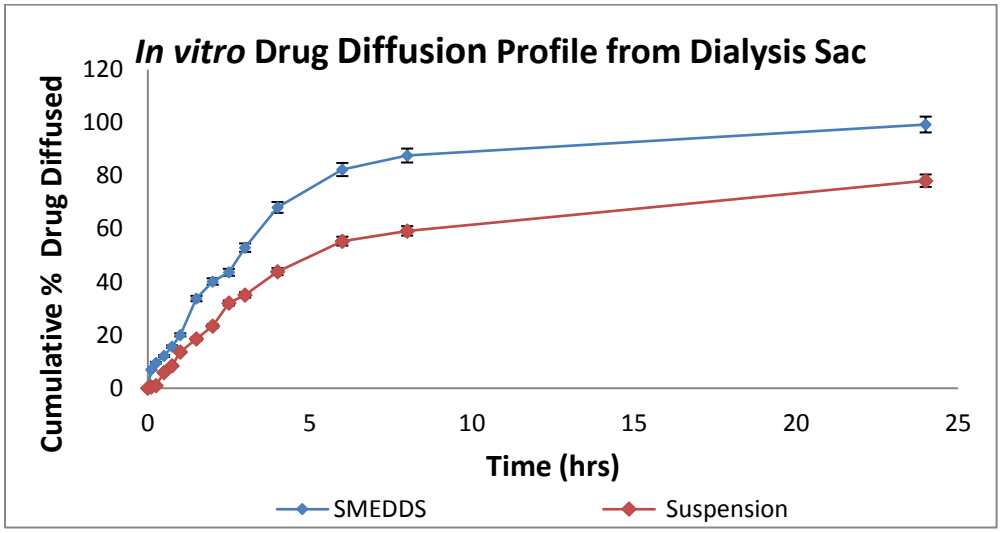


Fig. 7.3 *In Vitro* Drug Diffusion Profiles of SMEDDS and Drug

7.6.3 *Ex Vivo* Drug Permeability Study

Table 7.6 *Ex Vivo* Permeability Study of Optimized SMEDDS Formulation and Drug Suspension from Stomach

Sr. No.	Time (hrs)	% Cumulative drug release from Stomach ± Sd (n=3)	
		SMEDDS	Drug Suspension
1	0.25	8.694 ± 2.065	3.886 ± 0.698
2	0.5	16.368 ± 1.689	4.289 ± 1.231
3	0.75	31.259 ± 2.625	8.315 ± 1.896
4	1	39.625 ± 1.804	18.136 ± 0.359
5	1.5	47.68 ± 1.598	20.948 ± 1.247
6	2	59.488 ± 1.298	25.225 ± 1.634
7	3	65.93 ± 2.364	27.05 ± 1.348

After 24 hrs cumulative % drug released was 99.308 ± 1.203% and 78.328 ± 0.0698 % from SMEDDS and drug suspension respectively. The study shows that the diffusion profile of modafinil from dialysis sac was improved for SMEDDS due to more solubilization of drug by SMEDDS than the suspension. The higher amount of drug release in diffusion medium from SMEDDS may be due to difference in the volumes of solubilized drug. In addition, solubilized drug in SMEDDS was easily permeable through dialysis sac and thus improved the diffusion of modafinil. Study demonstrated that drug released from

SMEDDS was enhanced compared to suspension. *Ex vivo* drug permeability studies from isolated stomach for diluted modafinil SMEDDS and drug suspension are shown in Table 7.6 and Fig. 7.4. Within 3 hr of the time period show that more than 65% of the drug released from the stomach filled with diluted SMEDDS of modafinil which was approximately 2.5 times higher compared with the suspension. From the *ex vivo* study, it was inferred that absorption of the drug from the GIT can be enhanced with SMEDDS.

Table 7.7 *Ex Vivo* Permeability Study of Optimized SMEDDS Formulation and Drug Suspension from Intestine

Sr. No.	Time (hrs)	% Cumulative drug release from Intestine ± SD (n=3)	
		SMEDDS	Drug Suspension
1	0.25	16.326 ± 1.265	5.235 ± 2.365
2	0.5	26.326 ± 2.658	7.625 ± 0.986
3	0.75	36.598 ± 2.144	13.432 ± 1.658
4	1	49.658 ± 4.625	23.652 ± 1.964
5	1.5	61.268 ± 1.526	29.842 ± 1.196
6	2	69.634 ± 2.145	33.315 ± 2.658
7	3	81.236 ± 2.115	39.548 ± 2.042
8	4	89.745 ± 1.026	47.568 ± 1.794
9	6	94.341 ± 1.695	52.746 ± 1.025
10	8	97.482 ± 1.219	56.968 ± 0.865

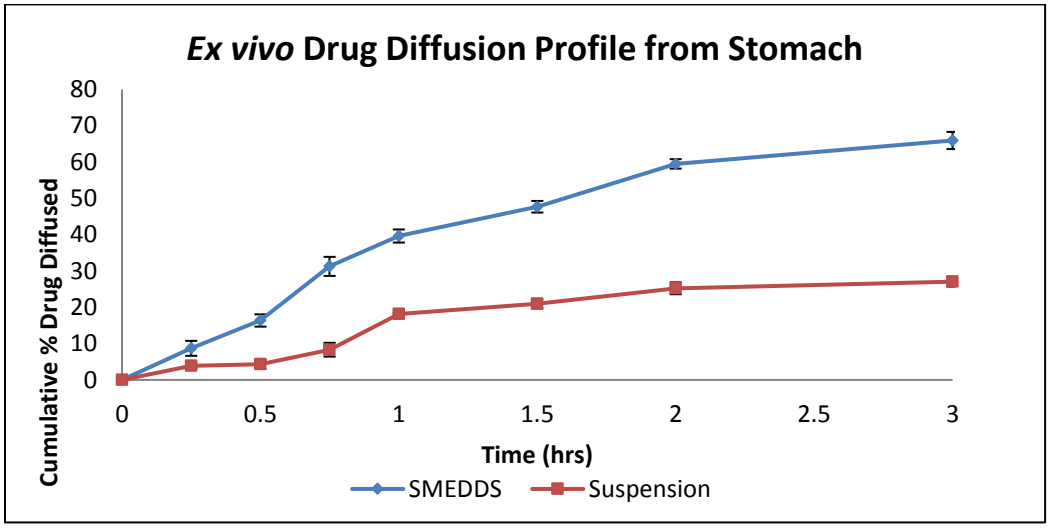


Fig. 7.4 *Ex Vivo* Drug Diffusion Profile of SMEDDS and Drug from Stomach

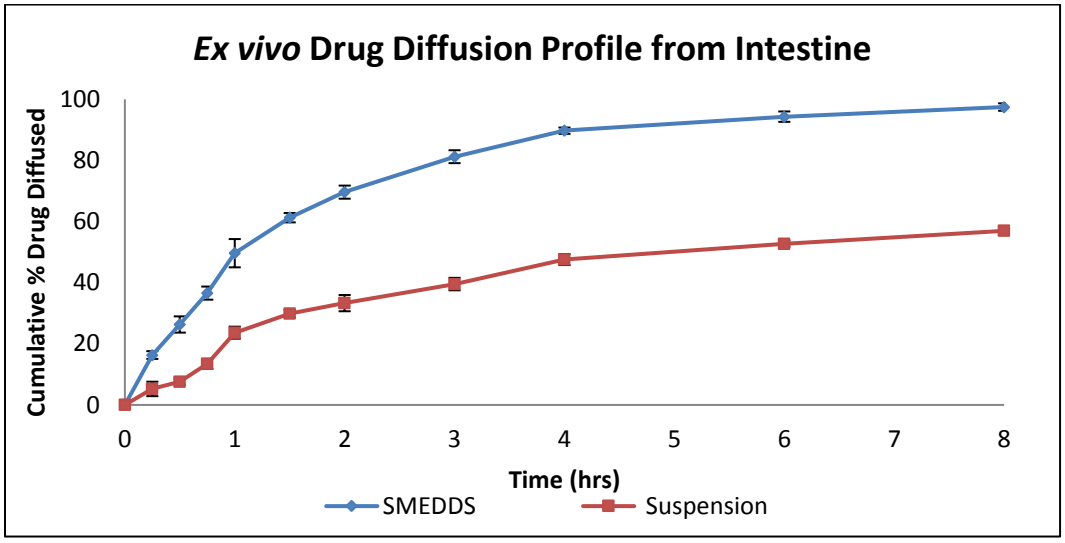


Fig. 7.5 *Ex Vivo* Drug Diffusion Profile of SMEDDS and Drug from Intestine

Ex vivo drug release studies from intestine (duodenum) for diluted Modafinil SMEDDS and drug suspension were shown in Table 7.7 and Fig. 7.5. It shows that within 8 hrs of the time period more than 90% of the drug released from the intestine filled with diluted SMEDDS of Modafinil which was approximately 1.7 times higher compared to suspension. Because of more solubilization of drug by SMEDDS, diffusion profile was improved. Thus, it can be inferred that the absorption of drug from GIT can be enhanced with SMEDDS, which hypothesized that finally enhancing the bioavailability.

SMEDDS shows that drug release was improved compared to drug suspension due to more solubilization of drug by SMEDDS formulation. Thus, from the intestine, the absorption of the drug can be improved with SMEDDS irrespective of the effect of intestinal pH for drug solubility. It was observed that the release of drug was found to be faster from the intestine in comparison to the stomach. The possible reason may be better absorption of drug from the intestinal region was due to the small globule size of microemulsion formed after administration of SMEDDS, high surface area of the intestine and more number of the absorption sites. Due to low solubility of the drug, its release from suspension was found to be very less both from stomach and intestine, while SMEDDS deals with already dissolved form of drug which easily diffused from GIT.

Chapter 7 (Part-B)

In Vitro & *Ex Vivo* Drug Release Study for NTB Microemulsion

Management of Dyslexia and ADHD

7.7 Introduction

7.7.1 *In Vitro* Drug Release

In vitro diffusion study of formulations is a valuable tool to predict behavior of a particular formulation with respect to drug transport across the membrane. *In vitro* model may have limitations in terms of prediction of drug transport across the mucosal membrane nevertheless, under the testing condition; moreover, *in vitro* studies can be helpful to access the relative drug transport behavior across mucosa. Various physicochemical parameters pertaining to formulations such as flux, partition coefficient, diffusion coefficient can be derived using *in vitro* evaluation techniques. Studies can also be used as a screening tool to screen the best formulation out of many. One of the disadvantages of *in vitro* evaluation techniques is that the method does not mimic the behavior of living tissues/organs. For example, degradation of drug compound in presence of enzymes, capricious blood supply or metabolism etc. In this study all the test formulations were accessed for *in vitro* diffusion across the sheep nasal mucosa in triplicate and physicochemical parameters were calculated. Understandably, for complex dosage forms like microemulsion *in vitro* release testing assumes greater significance. *In vitro* drug release studies are generally not only used in quality control of drug formulations, but also to predict the *in vivo* behavior as well as it can be used to check batch variability of a same formulation in drug release pattern. (1) The *in vitro* drug release of Vinpocetine formulations was performed by dialysis bag diffusion method.

7.7.2 *Ex Vivo* Drug Permeation Study

Study was performed on isolated tissue of the nasal cavity, outside the body in at most same environmental condition on tissue. *Ex vivo* conditions allow experiment under more controlled conditions than possible in the intact organism, at the expense of altering the "natural" environment. *Ex vivo* absorption study of formulations is a valuable tool to predict the behavior of a particular formulation with respect to drug transport across the nasal mucosa *in vivo*.(7, 8)

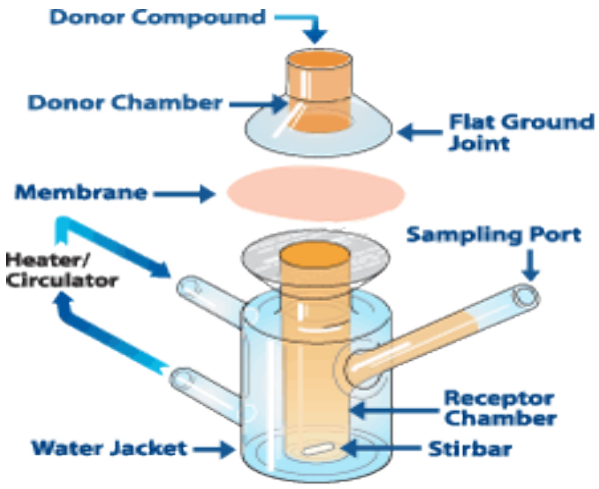


Fig. 7.6 Franz diffusion cell

The proposed *in vitro* studies were carried out using Franz diffusion cell (Fig 7.6). The Franz diffusion cell consists of a hollow glass tube in the center having diameter of 8mm. The cell has two compartments viz. (1) donor compartments and (2) receptor compartments. The donor compartment is used for holding the test formulation while the receptor compartment holds the respective diffusion media.

7.9 Materials and Instrumentation

Freshly prepared distilled water in the laboratory was used throughout the study. Sheep nasal mucosa was collected from the slaughter house. All the solutions required for study were prepared as per the Indian Pharmacopoeia, 2013.

Table 7.8 List of Materials and Reagents

Chemical/Reagents	Manufacturer / Supplier
Methanol	Astron chemical Ltd, Ahmedabad
Ortho phosphoric acid	S.D. Fine Chemicals, Vadodara

All other materials and reagents are same as describe in Table 7.1 of section 7.2

Table 7.9 List of Equipments

Equipments	Manufacturer / Supplier
Franz diffusion cell	V J Instruments, Mumbai
Dissolution Apparatus	USPXXIII apparatus I, Veevo, Mumbai
UV- Visible Spectroscophotoemeter-1700	Shimadzu Corporation, Kyoto, Japan

All other equipments are same as describe in Table 7.2 of section 7.2

7.10 *In Vitro* Drug Release Study by Diffusion Through Dialysis Bag

7.10.1 Activation of Dialysis Membrane (5)

The method remained same as discussed in 7.4.1 of section 7.4.

7.10.2 *In Vitro* Drug Release Studies

To elucidate the effect of microemulsion and mucoadhesive microemulsion systems on release kinetics of the drugs, *in vitro* release studies were performed for drug suspension, microemulsions, and mucoadhesive microemulsions using dialysis method. (9) Pretreated dialysis membrane (cellulose membrane, molecular weight cut-off was 12,000 Da, pore size 2.4nm) was soaked in simulated nasal fluid for 15 min. The drug release for suspension, ME and MME were estimated in dialyzing media; 10% methanolic phosphate buffer saline pH 6.4. One end of pretreated dialysis tubing was tied with thread, and then 1ml (5mg) of each formulation was placed in it along with 1 mL of dialyzing medium. The other end of the tubing was also secured with thread and was allowed to rotate freely in 20mL of dialyzing medium and stirred continuously at 100 rpm with magnetic bead on magnetic plate at 37°C± 0.5°C. Aliquots of 1mL were removed at different time intervals (15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540 min) and if required further diluted with diffusion media. Volume of aliquots was replaced with fresh dialyzing medium each time. These samples were analyzed quantitatively for the drug dialyzed across the membrane using UV-visible spectrophotometer against diffusion media as blank. The cumulative amount of drug released was calculated for the formulations and represented in Table 7.10 and shown graphically in Fig. 7.7. The kinetics of the drugs from the test formulations was evaluated by fitting the experimental data to different order kinetics such as zero-order, first order, and Higuchi's model. Each experiment was repeated three times.

7.11 *Ex Vivo* Drug Permeation Study by using Isolated Sheep Nasal Mucosa

Ex vivo drug permeation studies were performed using a Franz diffusion cell with a diameter of 12 mm and mucosa thickness approximately (height) 0.2 mm at 37.0 ± 0.5°C for 4hrs with stirring at 100 rpm. (10-12) The freshly excised sheep nasal mucosa was collected from the slaughter house in PBS pH 6.4. The membrane was identified and

separated from the nasal mucosa. The superior nasal conche was identified and separated from the nasal mucosa. (7) The excised superior nasal membrane was then mounted on Franz diffusion cell. The tissue was stabilized using 10 % methanolic phosphate buffer saline PH 6.4 in both the compartments and allowed to stir for 15 min on a magnetic stirrer. After 15 min, solution from both the compartments was removed and fresh 10 % methanolic phosphate buffer saline PH 6.4 was filled in the acceptor compartment. Nasal membrane mounting was done using glue at the brim of the donor compartment to avoid leakage of the test sample. The temperature of receiver chamber, containing 20 ml of diffusion media (10 % methanolic phosphate buffer saline PH 6.4) was controlled at 37±1° C under continuous stirring using Teflon coated magnetic bead at a constant rate (100 rpm), in a way that the nasal membrane surface just touches the diffusion fluid. Each study was carried for a period of 4 hrs, at predetermined time intervals of 15, 30, 45, 60, 90, 120, 180, 240 min; samples were withdrawn from the receptor compartment, replaced by an equal volume of diffusion media and withdrawn sample were analyzed by UV spectroscopy method. The experiment was performed in triplicate and data are shown in Table 7.11 and graphically represented in Fig. 7.8. The mean cumulative values for % drug release were calculated. The diffusion coefficient for the different formulations were calculated using following equation and tabulated in Table 7.12,

7.11.1 Determination of Diffusion Coefficient (D)

D = Kp * h / K

Where, **D** is the diffusion coefficient,

K is the octanol / PBS partition coefficient (3.9 for vinpocetine in octanol /water)

h is the thickness of the biological membrane (0.2cm)

Kp = Jss / Cd

Where, **Kp** represents permeability coefficient,

Jss is the steady-state flux = Slope of the graph of Cumulative permeation ($\mu g/h.cm^2$) vs Time (hr)

Cd is the initial concentration of drug in donor compartment = 4000 μg /cm

7.12 Result and Discussion

7.12.1 *In Vitro* Drug diffusion

Vinpocetine loaded ME, MME and suspension were subjected to *in vitro* release study through dialysis membrane for 9 hrs. Cumulative percentage drug diffuse were calculated and recorded in Table 7.10. Cumulative percentage drug diffuse vs time represented graphically in Fig. 7.7. Table 7.10 and Fig. 7.7 provide predictive approaches for diffusion pattern of Vinpocetine from ME, MME and Suspension. From suspension, only 18 % drug diffused after 9 hours. Whereas 62% and 71% of drug release from ME and MME respectively, show a significant ($p<0.05$) release of drugs at each time point with suspension.

Table 7.10 *In Vitro* Diffusion Profile of Vinpocetine loaded ME, MME and Suspension

Sr. No.	Time (min)	Cumulative % Drug Diffusion ± SD (n=3)		
		ME	MME	Suspension
1	15	9.23 ± 2.28	17.42 ± 1.33	2.38 ± 3.95
2	30	13.23 ± 1.31	22.42 ± 1.05	5.63 ± 2.28
3	45	17.73 ± 1.40	26.24 ± 1.84	9.21 ± 2.26
4	60	21.16 ± 1.39	30.13 ± 1.68	10.92 ± 3.05
5	90	23.94 ± 1.27	35.14 ± 1.21	11.18 ± 4.41
6	120	28.63 ± 1.46	39.15 ± 1.46	11.12 ± 3.20
7	180	30.99 ± 1.29	43.23 ± 1.64	12.68 ± 2.84
8	240	34.12 ± 1.85	46.64 ± 1.31	13.66 ± 3.64
9	300	38.11 ± 1.59	51.12 ± 1.42	14.12 ± 2.15
10	360	43.56 ± 0.49	58.08 ± 1.67	14.98 ± 2.69
11	420	50.74 ± 1.02	60.47 ± 0.89	15.39 ± 2.72
12	480	59.16 ± 1.55	65.86 ± 1.43	17.27 ± 3.31
13	540	62.43 ± 0.98	71.14 ± 1.11	18.31 ± 3.58

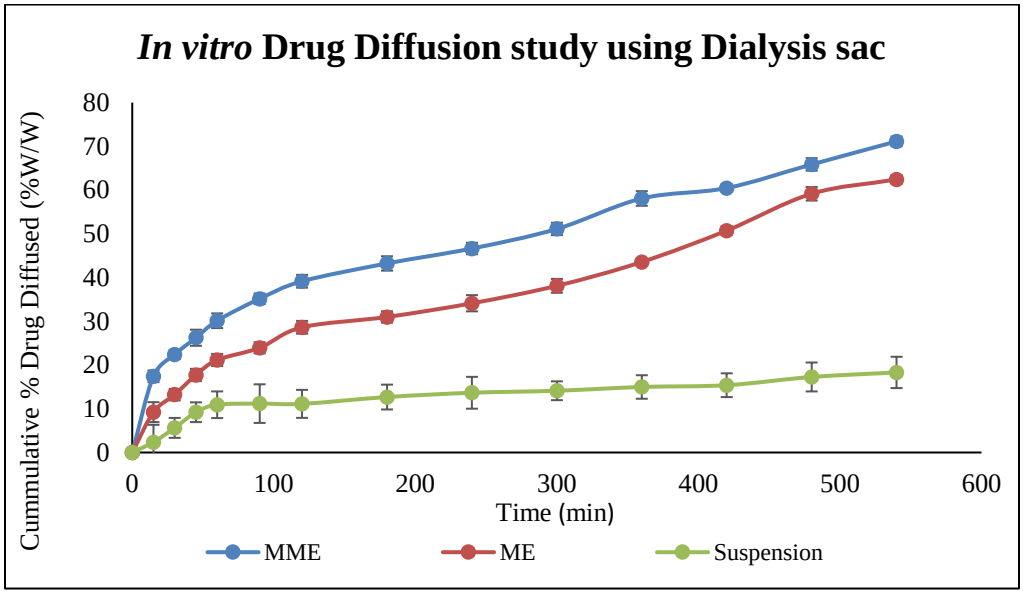


Fig. 7.7 *In Vitro* Diffusion Profile of Vinpocetine loaded ME, MME and Suspension

7.12.2 *Ex Vivo* Drug Permeability Study

Table 7.11 *Ex Vivo* Drug Permeation Study for Vinpocetine loaded ME, MME and Suspension

Sr. No.	Time (min)	Cumulative % Drug Release ± SD (n=3)		
		ME	MME	Suspension
1	15	06.23 ± 1.42	08.53 ± 1.63	2.35 ± 2.36
2	30	12.85 ± 1.49	15.69 ± 1.32	4.65 ± 1.86
3	45	19.17 ± 1.89	22.81 ± 1.21	7.43 ± 1.58
4	60	22.43 ± 1.09	27.37 ± 1.46	9.62 ± 1.94
5	90	26.19 ± 1.47	31.98 ± 2.48	10.84 ± 1.96
6	120	28.34 ± 1.43	34.89 ± 1.12	11.15 ± 2.68
13	180	32.51 ± 0.28	39.58 ± 1.94	12.54 ± 2.04
8	240	36.03 ± 0.94	43.39 ± 1.13	12.58 ± 1.94

Table 7.12 Diffusion Co-Efficient

Sr. No.	Formulation	Permeation Co-efficient (Cm^2/min)	Diffusion Co-efficient (Cm^2/min)	Regression coefficients (r^2)		
				Zero order	First order	Higuchi
1.	ME	2.76×10^{-5}	1.311×10^{-5}	0.783	0.966	0.968
2.	MME	3.35×10^{-5}	1.819×10^{-5}	0.815	0.985	0.991
3.	Suspension	1.10×10^{-5}	0.54×10^{-5}	0.769	0.813	0.881

Vinpocetine loaded ME, MME and Suspension were subjected to *ex vivo* drug permeation study from freshly excised sheep nasal mucosa for 4 hrs. Cumulative percentage drug diffuse was calculated against time presented in Table 7.11 and Fig. 7.8. Approximately 32% and 39 % drug released from ME and MME within 4 hrs, while only 12% drug release from suspension. This result show that the drug release from the suspension was significantly different from that of ME and MME. The drug loaded within oil droplet with external aqueous phase performed in favor of Vinpocetine permeability. ME could act as drug reservoir and drug loaded in ME is released from internal phase to external phase. On the other hand, chitosan in MME act as permeation enhancer as well mucoadhesive agent and give a significant release of drug both from ME and suspension.

The decreasing order of diffusion coefficient for the formulations was Suspension < ME < MME shown in table 7.12. The results revealed that the ME and MME formulations successfully diffused through nasal mucosa and among all three formulations, the mucoadhesive microemulsion, the drug exhibited highest diffusion coefficient and better drug release profile, whereas it was least for Suspension. Microemulsion showed better diffusion coefficient compared to suspension. The results of our study are in accordance with similar reports available in literature. This may be due to the transcellular uptake, high solubilization capacity, as well as the potential for enhanced absorption of microemulsion system. Mucoadhesive microemulsion formulated with the aid of chitosan, which act as a permeation enhancer and/or open the tight junction of nasal epithelium. MME exhibited higher diffusion due to the presence of mucoadhesive agent that probably adhere to mucosa thereby increased contact and hence increased diffusion. Improved drug diffusion profile, this result suggests that, lipophilic permeation enhancer Capmul MCM C8 oil as well surfactant and co-surfactant can be a useful tool to improve the membrane permeability in the nasal delivery of lipophilic drug using ME as drug delivery system. Kinetic modeling of diffusion of drug formulation exhibited higher R^2 values for the highuchi model compared with zero and first order. This may occur because the diffusion system has a reservoir compartment (donor compartment) and nasal mucosa acts as a barrier; therefore, the diffusion will mimic and shall be closer to reservoir system than zero order (concentration independent) or first order (concentration gradient) diffusion.

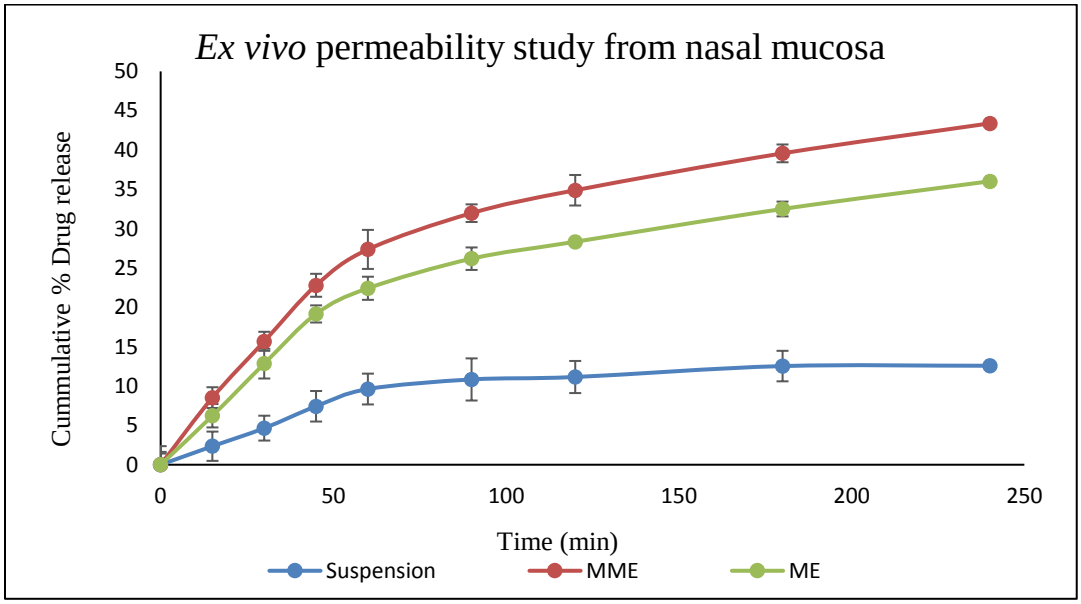


Fig. 7.8 *Ex Vivo* Drug Release of Vinpocetine loaded ME, MME and Suspension

Diffusion profile was improved for ME and MME due to more solubilization of drug. MME showed better release profile compared to ME formulations due to presence of permeation enhancer in formulation. Thus, it can be inferred that diffusion of the drug from the nasal mucosa can be enhanced with MME which may lead to increase in bioavailability.

7.8 References

1. D'Souza S. A review of in vitro drug release test methods for nano-sized dosage forms. Advances in Pharmaceutics. 2014;(2014), 1-12
2. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. European journal of pharmaceutical sciences. 2001;13(2):123-33.
3. Prajapati ST, Joshi HA, Patel CN. Preparation and characterization of self-microemulsifying drug delivery system of olmesartan medoxomil for bioavailability improvement. Journal of pharmaceutics. 2012;(2013)1-9
4. Bhagwat DA, D'Souza JI. Formulation and evaluation of solid self micro emulsifying drug delivery system using aerosil 200 as solid carrier. International current Phaarmaceutical journal. 2012; 1 (12), 414-9.
5. Sigma. Aldrich. Product information Dialysis tubing, cellulose membrane. Louis: USA.

6. Dixit AR, Rajput SJ, Patel SG. Preparation and bioavailability assessment of SMEDDS containing valsartan. AAPS pharmscitech. 2010;11(1):314-21.
7. Patel RB, Patel MR, Bhatt KK, Patel BG. Formulation consideration and characterization of microemulsion drug delivery system for transnasal administration of carbamazepine. Bulletin of Faculty of Pharmacy, Cairo University. 2013;51(2):243-53.
8. Patel RB, Patel MR, Bhatt KK, Patel BG. Formulation and evaluation of micro-emulsion based drug delivery system for intranasal administration of olanzapine. Int J Biomed Pharm Sci. 2013;7(1):20-7.
9. Rajput Amarjitsing Premsinh, Patil Vikas P, Chaudhari P M, Chaudhari S P, T BD. Nose to brain delivery of Ziprasidone microemulsion: Design and Characterization. International Research Journal of Pharmacy. 2013; 4(7):170-177.
10. Naik A, Nair H. Formulation and evaluation of thermosensitive biogels for nose to brain delivery of doxepin. BioMed research international. 2014;(2014) : 1-10
11. Pathak R, Dash RP, Misra M, Nivsarkar M. Role of mucoadhesive polymers in enhancing delivery of nimodipine microemulsion to brain via intranasal route. Acta Pharmaceutica Sinica B. 2014;4(2):151-60.
12. Kumar A, Sharma P, Chaturvedi A, Jaiswal D, Bajpai M, Choudhary M, Yadav IK, Singh HP, Chandra D, Jain D. Formulation development of sertraline hydrochloride microemulsion for intranasal delivery. Int J ChemTech Res. 2009;1(4):941-7.