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Self Nanoemulsifying Drug Delivery System of Olanzapine for Enhanced Oral Bioavailability: *In vitro*, *In vivo* Characterisation and *In vitro* -*In vivo* Correlation

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Abstract

Lipid based self nanoemulsifying drug delivery system (SNEDDS) was explored to improve the oral bioavailability of olanzapine (OLZ), a poorly water-soluble drug candidate, using spontaneous emulsification method. Nanoemulsions have ability to enhance the oral bioavailability of poorly water soluble or lipophilic drugs through selective lymphatic pathways. Following optimization, (from pseudo ternary phase diagram) OLZ SNEDDS consisting of Capryol 90(36.2%), Brij 97(14.6%) and ethanol (42.5%) were selected. The globule size (90 nm), and polydispersity index (0.287), was found to be minimum. The pharmacokinetic study was conducted on rabbits and the parameters like peak concentration (C_{max}), time of peak concentration (T_{max}), etc. were evaluated by Wagner nelson method. The *in vivo* studies concluded that there was 1.2 fold and 1.6 fold increase in bioavailability of nanoemulsion when compared with marketed tablet formulation and drug suspension, respectively. This may be attributed to increased solubility and enhanced permeability of the drug from nanosized emulsion. From the similarity factor between biorelevent dissolution media and 0.1 N HCl (pH 1.6) it was concluded that the 0.1 N HCl (pH 1.6) can be used for the dissolution of SNEDDS to predict the *in vivo* bioavailability instead of the biorelavent media. The level A correlation with correlation factor 0.97 was achieved, which showed that there is a good correlation between *in vitro* dissolution and *in vivo* bioavailability and the dissolution studies can be used as a surrogate for the *in vivo* studies.

Keywords: Olanzapine; SNEDDS; Biorelavent dissolution media; IVIVC

Introduction

BCS Class II drugs suffer from poor water solubility and high lipophilicity resulting in a highly variable oral bioavailability. Even though they contain potential pharmacodynamic activity they fail to reach market. Bioavailability of Class II drugs is rate limited by its dissolution. Hence a slight increase in its dissolution rate results in a large increase in bioavailability. The solubility of the drug could be increased in three ways: changing the chemical structure in the lead optimization phase; prodrug approach and the formulation approach. Formulation strategies such as micronization, co-solubulization, solid dispersion, inclusion complex, nanosuspension, lipid based formulations etc., may be employed to enhance their dissolution, thereby their bioavailability [1]. Products of micronization and nanosuspension are thermodynamically unstable. A method such as co-solubulization, solid dispersion, and inclusion complex formation, involves altering of the physical property of the drug, which is not desirable. Lipid based drug delivery systems (LBDDS) are gaining importance these days due to their ability to deliver drug via lymphatic route restraining the hepatic metabolism. LBDDS are a diverse group of formulations which are classified into 4 types: Type I (oils without surfactants), Type II (oils with water insoluble surfactants), Type III (oils, surfactants, co solvents), Type IV (oils with water soluble surfactants and co-solvents). Of which, Type III popularly known as Self Nano Emulsifying Drug Delivery Systems (SNEDDS) are widely used because of their ease of formulation: simple self-emulsification technique [2]. SNEDDS are defined as isotropic mixtures of natural or synthetic oils containing solid or liquid surfactants and one or more hydrophilic solvents. They form fine oil in water (o/w) emulsions on contact with GI fluids. Usually the drug in SNEDDS remains in the solution form throughout its GI transit time whereby they circumvent the dissolution step. It involves digestion of the excipients and formation of different colloidal structures as nanodroplets. The drug gets partitioned into these structures before it is absorbed. More over the nanodroplets formed are with increased surface area due to decreased interfacial tension. Thus they are readily available for absorption of poorly soluble drugs [3]. In addition to increased surface area the common excipients used in the formulation of SNEDDS inhibits P-gp & CYP450 enzymes thereby decreasing intestinal efflux and drug biotransformation respectively [4]. Research also reveals that SNEDDS facilitates the transcellular and paracellular absorption thereby the drug is absorbed through the lymphatics via chylomicron sysnthesis of the fatty components of the oil phase of the emulsion [5].

The main objective of the study is to formulate SNEDDS of one of the BCS Class II drug, Olanzapine, and to perform *in vitro* characterization studies, *in vivo* bioavailability studies, and to develop IVIVC. Olanzapine is a potent muscarinic M_3 receptor antagonist widely used for its antipsychotic effects. Steady state concentration of the drug is necessary in the plasma for its effective pharmacological action. Though the present marketed formulations do not suffer from the lack of steady state plasma levels of the drug, the conventional formulations require high doses of the drug than needed to obtain such concentrations due to first pass metabolism [6]. Hence suggesting SNEDDS for formulating olanzapine improves bioavailability and

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thereby decreases the dose of the drug. Due to the formation of colloidal structures of SNEDDS after its ingestion simple *in vitro* dissolution testing is not enough to predict its dissolution behavior *in vivo*. Hence biorelevant media has been developed to represent both the pre and post prandial states in the proximal gut region. The composition of the media has been fine tuned to mimic the phase of digestion both in the stomach and the upper small intestine. Thus the exact rate of drug release from the dosage form could be assessed; the effect of food on *in vivo* exposure of drug can also be predicted [7]. IVIVC as defined by USFDA is a predictive mathematical model describing the relationship between the *in vitro* property (rate or extent of drug dissolution or release) of an oral dosage form and the relevant *in vivo* performance (plasma drug concentration or amount absorbed) [8,9]. It is desirable to establish Level A correlation where point to point correlation exists between *in vitro* absorption and *in vivo* release profile. IVIVC developed leads to improved product quality and reduces regulatory burden [10]. IVIVC is used as a method for evaluating product quality as well as a surrogate method for predicting the biological performance of a dosage form *in vivo* [11]. Predictive and reliable IVIVC forms the basis for biowaiver, allowing reduction in time and cost of production [12].

Materials and Methods

Materials

Olanzapine drug was a gift sample from Orchid chemicals & Pharmaceuticals Ltd., Chennai; Bile salts LR, Ethanol, Sodium Chloride Hi pure, Lecithin, Capryol 90, Brij 97, Pepsin, Hydrochloric acid LR, Potassium dihydrogen ortho phosphate, Sodium hydroxide pellets, Potassium bromide (IR grade), and Triethylamine, Acetonitrile HPLC grade, Ortho Phosphoric acid, Sodium citrate LR and Methanol HPLC grade were purchased from Rankem Pvt Ltd.,.

Methods

Solubility studies [13]: Solubility of Olanzapine was determined in various oils such as Capryol 90, Maisine 35-1, Arachis oil, Linseed oil, by shake flask method described by Sheikh Shafiq-Un-Nabi et.al. The drug dissolved in the oil was extracted with chloroform as a solvent and the amount of drug (Tables 1 and 2) was determined using HPLC at 254nm.Solubility of Olanzapine in various dissolution media like distilled water, 0.1N Hcl, pH 6.8 and 7.4 phosphate buffer, and biorelevant media. (Compatibility studies were performed using FTIR 8400 S, Shimadzu. The IR spectrum of the physical mixture was compared with those of pure drug, lipid and surfactants and peak matching was done to detect any appearance or disappearance of peaks).

SNEDDS were formulated by spontaneous emulsification technique using slow aqueous titration method [14].

Construction of pseudo ternary phase diagrams: Pseudo ternary phase diagrams were constructed to examine the formation of oil in water nanoemulsion with 4 components oil, surfactant, co surfactant, and aqueous phase. The 4-component system consisted of (i) Capryol 90 (Selected from solubility studies) (ii) surfactant Brij 97 with HLB value 12.5 (iii) a Co surfactant (Ethanol) and (iv) distilled water (aqueous phase). Surfactant and co surfactant mixture (SCoS) in each group were mixed in different weight ratios (1:0, 1:1, 1:2, 1:3, 1:4, 2:1, 3:1, 4:1). Seventeen combinations of oil and SCoS, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1 were made so that maximum ratios were covered for the study to depict the boundaries of phases precisely formed in the phase diagrams Figures 1-5. Slow titration with aqueous phase was done to each weight ratio of oil and SCoS and visual observation was carried out for transparency and flowability of the nanoemulsion.

Different concentrations of oil from NE region of phase diagrams were selected at a difference of 5% and the drug is incorporated. For each 5% of oil selected, the formula that used the minimum concentration of SCoS for its NE formulation was selected. For convenience 1ml was selected as the NE formulation. 2.5 mg of Olanzapine was selected as the dose to be incorporated into the oil phase where the oil phases completely solubulized the drug [13].

Characterisation of nanoemulsion:

Thermodynamic stability studies and dispersibility tests (15): The formulations were subjected to different thermodynamic stability studies (Table 3) such as centrifugation, heating-cooling cycle and freeze thaw cycle, to avoid the selection of metastable formulations. The dispersibility of the nanoemulsion was assessed using a standard USP XXII dissolution apparatus II. The *in vitro* performance of the

OIL	SOLUBILITY(mg/ml)
Capryol 90	41.2±1.98
Maisine 35-1	32.1±0.99
Linseed oil	19.3±0.81
Arachis oil	9.04±1.22
Castor oil	4.23±0.51

Table 1: Solubility of Olanzapine in various oils.

SOLVENT	SOLUBILITY
0.1 N HCl	90.3 ± 2.3 mg/ml
Phosphate buffer pH 6.8	173 ± 1.7 µg/ml
Phosphate buffer pH 7.4	145.4 ± 4.1 µg/ml
Distilled water	43.4 ± 1.74 µg/ml
Biorelevant media	93.1 ± 3.1 mg/ml

Table 2: Solubility of drug using HPLC.

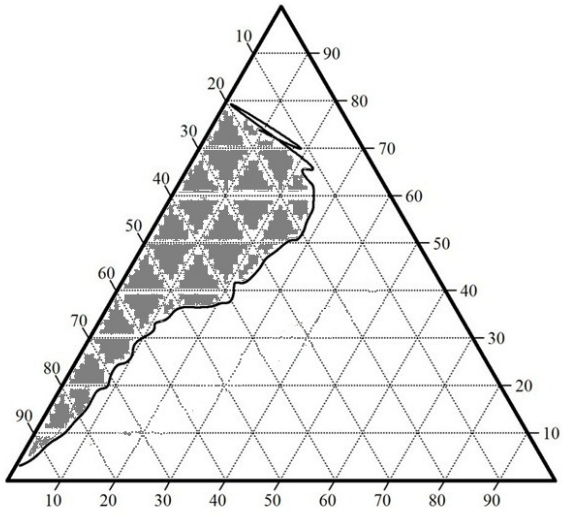

Figure 1: Capryol 90 (Selected from solubility studies).

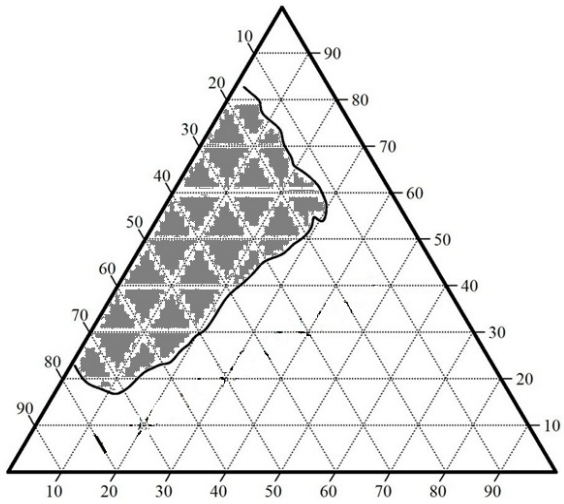

Figure 2: Surfactant Brij 97 with HLB value 12.5.

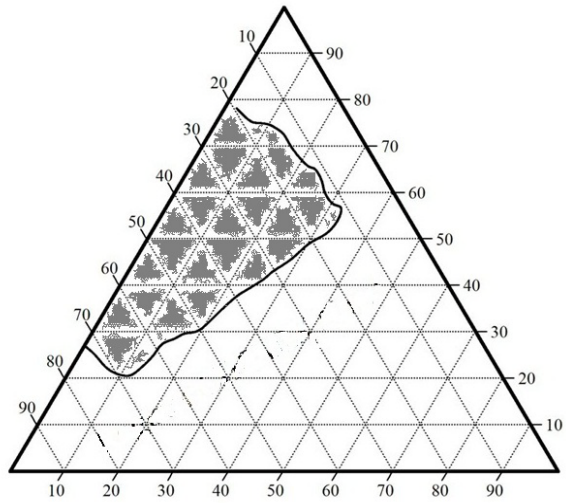

Figure 3: A Co surfactant (Ethanol) phase diagram.

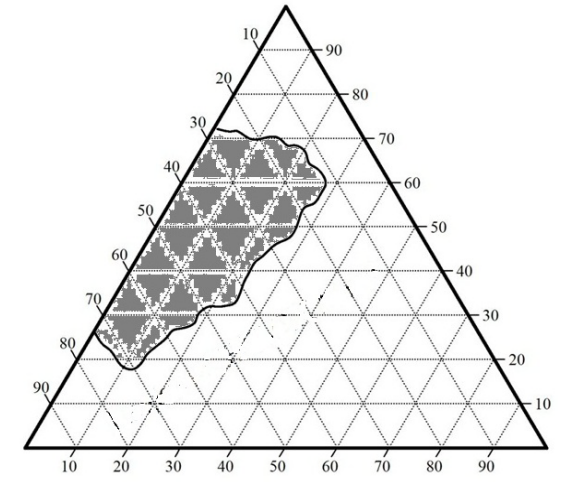

Figure 4: Distilled water (aqueous phase) diagram.

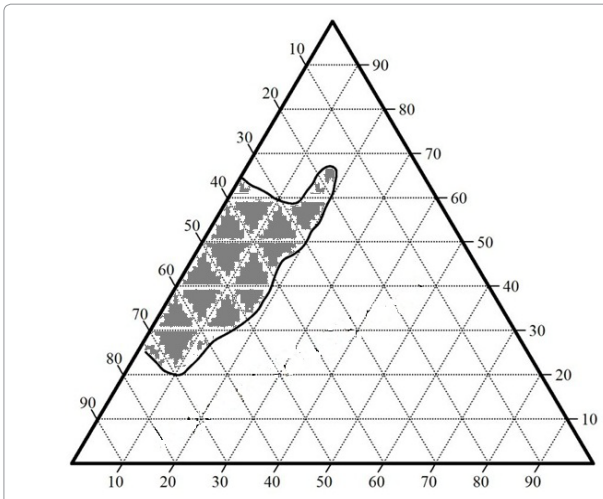

Figure 5: Construction of pseudo ternary phase diagram.

SCoS	Oil %	ScoS %	Aq %	Centrifugation	H/C cycle	Freeze Thaw	Dispersion
1:0	10	30	60	F	P	P	-
	15	56	29	P	F	F	-
1:1	10	18	72	P	P	P	-
	15	25	60	P	P	P	-
	20	36	54	P	P	P	-
	25	45	30	P	F	P	-
1:2	10	27	63	P	F	P	-
	15	32	53	P	P	F	-
	20	37	43	P	P	F	-
	25	44	31	P	P	P	***
	30	56	14	P	P	P	***
1:3	10	18	72	P	F	F	-
	15	19	66	P	P	P	-
	20	30	50	P	P	P	-
	25	36	39	P	P	F	-
	30	51	19	P	P	P	***
1:4	10	18	72	P	P	P	-
	15	21	64	P	P	P	-
	20	35	45	P	P	P	-
2:1	10	22	68	P	P	F	-
	15	25	60	P	P	P	-
	20	33	47	P	P	P	***
	25	40	35	P	P	P	-
	30	45	15	P	P	P	-
3:1	10	18	72	P	P	P	-
	15	25	60	P	P	P	***
	20	32	48	P	P	F	-
4:1	10	20	70	P	P	P	***
	15	25	60	P	P	P	-

***formulation which passed the dispersibility test; P- pass; F- fail.

Table 3: Thermodynamic stability studies and dispersibility tests.

formulations was visually assessed by phase clarity, self-emulsification time, and rate of emulsification.

Rapidly forming (within 1min) nanoemulsion, having a clear or bluish appearance, rapidly forming, slightly less clear emulsion, having a bluish white appearance were selected for further studies (Table 3). The selected formulations were prepared by dissolving 2.5 mg (single dose) of Olanzapine in oil (10%, 15%, 20%, 25% etc.). Respective SCoS ratio was added to the oil, and mixed using vortex mixer.

The formulated SNEDDS were evaluated for the following parameters *in vitro* (Table 4) [15,16].

From the stock solution of Olanzapine, dilutions were prepared and the calibration curve in 0.1N Hcl and biorelevant media constructed.

Chromatographic conditions for Olanzapine:

The following is the optimized chromatographic conditions, selected for the estimation of Olanzapine in dissolution samples with Atorvastatin as internal standard. Shimadzu gradient HPLC system was used with following configuration: LC-20 AD 230 V Solvent delivery system (Pump); Manual Injector 25 µl (Rheodyne); SPD-M20A 230 V Photo diode array detector; Stationary phase: Phenomenex Gemini C18 (250×4.6 mm i.d., 5 µ; Mobile phase: Acetonitrile: 25 mM Potassium dihydrogen orthophosphate (pH 6.5); Mobile phase ratio: 40:50; Flow rate**:** 1ml/min; Sample volume**:** 20 µl; Detection: 254nm; Data station: LC solutions.

***In vitro* drug release studies:** *In vitro* drug release in 0.1 N Hcl and biorelevant media:

The quantitative *in vitro* release test [17] was performed in 900 ml 0.1 N HCL and biorelevant media using USP dissolution apparatus Type I at 50 rpm at 37 ± 0.5°C. The Optimized SNEDDS formulation containing single dose (2.5mg) of Olanzapine was filled in Size 3cs of Hard gelatin capsule [18] (CONISNAP). Samples were withdrawn at regular time intervals. The release of drug from SNEDDS formulation was compared with the conventional tablet formulation and the samples were analyzed for the drug content using HPLC at 254 nm (Table 5).

A simple dissolution media doesn't meet the requirements to predict the dissolution of SNEDDS *in vitro*. Hence biorelevant media was developed which physiologically represent the *in vivo* biological environment in composition (Table 6) [8].

***In vivo* bioavailability studies [18]:** The experiments were carried out after getting the approval of the CPCSEA and IAEC, JSS College of Pharmacy, Ooty. Proposal no: JSSCP/IAEC/M.PHARM/PH.CEUTICS/01/2010-11 and their guidelines were followed throughout the experiment.

Formulation	A	B	C	D	E
ScoS(ml)	1:2	1:3	2:1	3:1	4:1
Oil %	25	30	20	15	10
S%	14.5	12.7	22	18.7	16
Cos%	29.3	38.2	11	6.25	4
Aq%	31	19	47	61	70
Globule size(nm)	90	254.3	212	40	-
PDI	0.287	0.723	0.398	0.910	-
Zeta Potential(mV)	-19.0	-17	-22	-5	-
Viscosity(cP)	22.3	30.1	19.2	-	-
Conductivity (µS/cm)	451.3	-	522.3	-	-
Refractive Index	1.49	-	1.27	-	-
Transmittance	99.43	-	98.9	-	-

S. No.	PARAMETER	METHOD
1	Globule size & polydispersity index	Zetasizer 3000 (Malvern Instruments Worcestershire, UK) (Figure 6)
2	External Morphological Study	Scanning Electron Microscopy (Figure 7)
3	Viscosity determination	Brookfield DV-II ultra+ viscometer
4	Electro conductivity study	Electro conductometer (Conductivity meter 305, Systronic).
5	Refractive index and percent transmittance	Abbe refractometer & UV spectrophotometer
6	*In vivo* performance	50,100, 1000 times dilution with various dissolution media viz: 0.1N Hcl (pH 1.2) and biorelevant media.

Table 4: The formulated SNEDDS *in vitro*.

Time (min)	SNEDDS (% cumulative release)		Marketed formulation (% cumulative release)	
	0.1 N HCl	Biorelevant media	0.1 N HCl	Biorelevant media
15	33.17	38.21	32.33	29.9 1
30	56.28	64.61	49.49	45.18
45	72.41	75.37	62.32	57.42
60	79.37	81.15	73.39	70.36
90	93.23	95.37	75.00	74.10
120	96.64	98.95	75.30	74.20

Table 5: *In vitro* drug release in 0.1 N Hcl and biorelevant media.

COMPOSITION	STRENGTH
Bile salt	80 µm
Lecithin	20 µm
Pepsin	0.1 mg/ml
Sodium chloride	34.2 mM
Hydrochloric acid	Qs
pH	1.6

Table 6: The *in vivo* biological environment in composition.

Estimation of drug in rabbit plasma: Healthy overnight fasted New Zealand white rabbits were used for the experiments. The animals were not anaesthetized during or prior to the experiment. The animals were given water *ad libitum* during fasting and throughout the experiment. Zero hour fasting blood samples were withdrawn early in the morning. The animals were then divided into 3 groups each containing two animals. The dose for the rabbits was selected based on the surface area ratio of rabbit and man and the dose was administered with an oral cannula. Group 1 received drug suspension (0.3% sodium CMC), group 2 received conventional marketed tablet formulation and group 3 received SNEDDS. Immediately after administration the animals were given 5 ml of water.

Blood samples (0.5 ml) were withdrawn from the marginal ear vein at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12 hours with a sterile syringe. The blood samples were collected in a ria vial containing the anti-coagulant (0.4 ml of 2.5% sodium citrate), centrifuged at 2500 rpm for 4 min and the plasma samples were separated and stored at -20°C. The plasma samples were deprotonated and extraction of drug was done by solid phase extraction (SPE) using ACN-water mixture and analyzed. Estimation of plasma samples by HPLC was carried out using optimized chromatographic conditions mentioned earlier (Table 7).

Data analysis: Predicting the release behavior of the drug *in vitro* was done by pharmacokinetic treatment of data. Pharmacokinetic parameters [19] (AUC, AUMC, $T_{1/2}$, Fr, MRT etc.) (Table 8) after oral administration of Olanzapine in rabbits were calculated. The pharmacokinetic data was analyzed by one-way analysis of variance (ANOVA) using Tukey-Kramer method (Table 8) [19].

***IVIVC* development:** IVIVC is based on a mathematical model that relates the fraction of drug released *(in vitro)* to the fraction of total drug absorbed across the gastrointestinal mucosa *(in vivo)*. The main objective of developing and evaluating IVIVC is to enable the

dissolution test to serve as a surrogate for *in vivo* bioavailability studies. This reduces the number of bioequivalent studies required for approval. Level A IVIVC [20] represents a point-to-point correlation between the *in vivo* absorption profile and the *in vitro* release profile (Figures 8 and 9). The *in vivo* absorption profile is calculated from plasma concentration–time curves using Wagner-nelson method. From this the fraction of drug absorbed *in vivo* is correlated with the fraction of drug dissolved *in vitro*. The fraction of drug dissolved *in vitro* can be calculated from *in vitro* drug release. The fraction of drug absorbed can be calculated by the formula.

$$F_a = (C_p)t + +k(AUC)_{o \to t}$$

Results and Discussion

A. Solubility studies of olanzapine in different oils

Solubility is an important criterion in formulation of SNEDDS, as the drug remains in the liquid form solubulized in the oil phase. Hence the oil phase in which the drug shows maximum solubility is to be selected for the purpose. From Table 1 it was evident that Capryol 90 shows maximum solubility of Olanzapine i.e., 41.2 ± 1.98 mg/ml. Hence Capryol 90 was selected for the formulation of SNEDDS.

Time (hrs)	Pure drug suspension (ng/ml)	Marketed Formulation(ng/ml)	SNEDDS (ng/ml)
0.25	19.15 ± 0.81	30.27 ± 1.21**	48.25 ± 1.90**##
0.5	25.20 ± 0.98	40.14 ± 1.75**	69.15 ± 2.31***##
0.75	34.89 ± 1.09	48.80 ± 1.92**	78.13 ± 2.96***##
1	53.6 ± 1.33	57.14 ± 1.98	80.25 ± 2.34**##
2	56.42 ± 1.89	60.65 ± 2.13	76.47 ± 2.28**##
4	40.55 ± 1.33	57.85 ± 1.94**	69.75 ± 1.99**#
6	35.46 ± 1.16	48.90 ± 1.89**	60.43 ± 1.94**
8	30.16 ± 0.98	40.38 ± 1.71*	50.97 ± 1.78**
12	24.39 ± 0.95	29.79 ± 1.54**	32.18 ± 1.49**

Values are expressed as mean ± S.D; n=2
*P<0.05; **P<0.01;***P<0.001 when compared with pure drug
#P<0.05; ##P<0.01 when compared with conventional formulation
One way ANOVA followed by TUKEY-KRAMERS multiple comparision tests.

Table 7: Estimation of plasma samples by HPLC.

Pharmacokinetic parameters	Drug suspension	Marketed Tablet formulation	SNEEDS
T_{max}(h)	2	2	1
K_e	0.08095 ± 0.0266	0.0957 ± 0.0153	0.115 ± 0.0162
C_{max}(ng/ml)	56.42 ± 1.89	60.65 ± 2.13	80.25 ± 2.34**##
$T_{1/2}$(h)	8.56 ± 0.21	7.242 ± 0.15	6.026 ± 0.18**##
$AUMC_{0-t}$(ng h/ml)	31417.76 ± 1920	49855.41 ± 1860	75655.37 ± 2410**##
$AUMC_{0-\infty}$ (ng h/ml)	149156.8088 ± 490	218219.4643 ± 530	276444.5874 ± 610**##
AUC_{0-t}(ng h/ml)	473.22 ± 35.52	550.71 ± 42.56*	691.3738 ± 68.9**##
$AUC_{0-\infty}$ng h/ml)	738.527 ± 72.5	861.99 ± 21.6	971.199 ± 59.35**##
F_r	----	1.3	1.6
F_r	----	----	1.2
MRT(h)	201.965	253.158	284.642

Values are expressed as mean ± S.D; n=2
*P<0.05; **P< 0.01;***P<0.001 when compared with pure drug
#P<0.05; ##P<0.01 when compared with conventional formulation
One way ANOVA followed by TUKEY-KRAMERS multiple comparision test.

Table 8: The pharmacokinetic analysis of variance using Tukey-Kramer method.

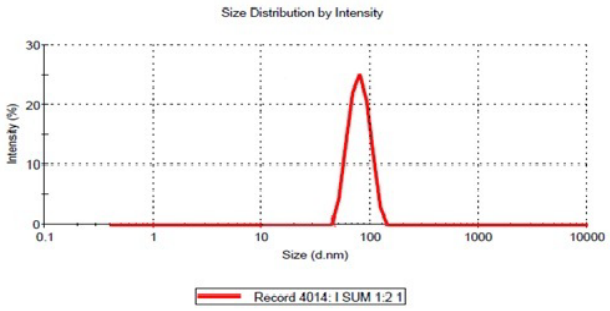

Figure 6: Size distribution by intensity.

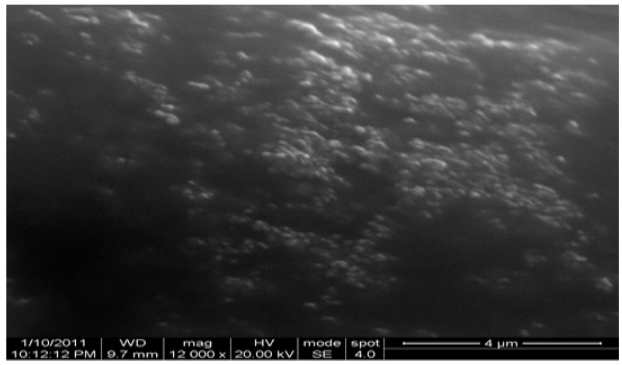

Figure 7: The scanning electron microscopic study on the external morphology of the nanoparticles.

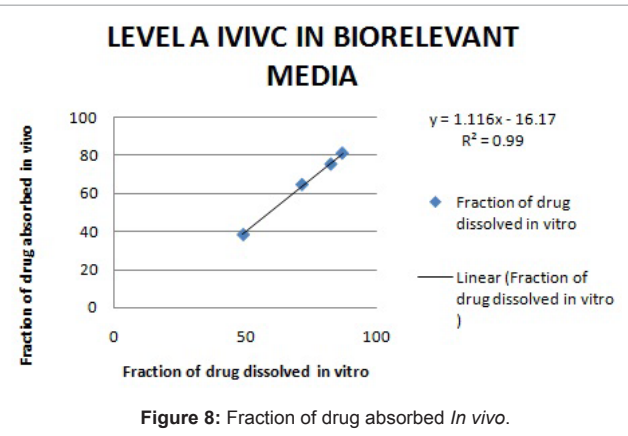

Figure 8: Fraction of drug absorbed *In vivo*.

B. Solubility studies of olanzapine in different dissolution media

From Table 2, Olanzapine was found to be highly soluble in 0.1 N HCl (90.3 ± 2.3 mg/ml) and biorelevant media (93.1 ± 3.1 mg/ml). The drug shows a slight increase in the solubility in biorelevant media. It can be assumed that the biorelevant media influences the dissolution of the drug and hence dissolution studies are to be performed in biorelevant media.

Pseudo ternary phase diagram study

Phase behavioral studies were performed by constructing phase

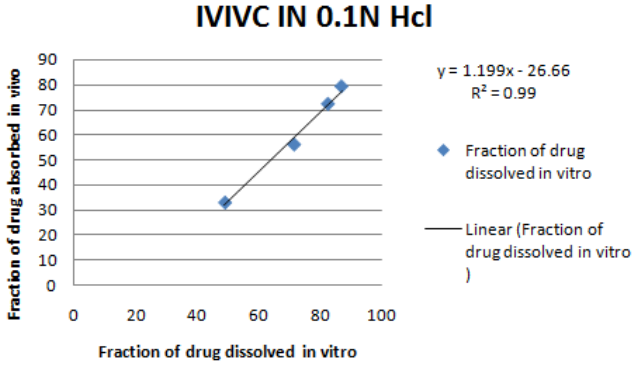

Figure 9: Fraction of drug dissolved *In vitro*.

diagrams that depict the boundaries of different phases, as a function of composition and temperature, to investigate the structural organization of the emulsions formed [21]. When surfactant alone (ScoS 1:0) was used only a small area of nanoemulsion is formed with oil solubulized upto 18% and ScoS 65%, while surfactant and co surfactant were taken in equal ratio (ScoS 1:1) there was a slight increase in nanoemulsion region but the oil solubulization increased upto 23% with 59% of ScoS. This could be due to the addition of co-surfactant which leads to greater penetration of oil phase into the surfactant thereby decreasing the interfacial tension.

When the concentration of co-surfactant is doubled (SCoS 1:2) nanoemulsion area increased considerably with 30% oil solubulized with 55% SCoS. There was no difference in nanoemulsion region and the oil solubulization remained same for (SCoS 1:3). But on further increase of co-surfactant (SCoS 1:4) the oil solubulization decreased to 28%.

When Surfactant concentration (SCoS 2:1) was doubled nanoemulsion area was large and the maximum oil solubulization was up to 32% with only 52% of SCoS. A further increase in surfactant concentration i.e., SCoS 3:1 resulted in decrease of oil solubulization of only up to 25%. For SCoS 4:1 even a high concentration of SCoS 65% resulted in less nanoemulsion area with only 19% of oil solubulization.

Thermodynamic stability studies and dispersibility tests

The formulations selected from the ternary phase diagrams were subjected to different thermodynamic stability studies and the formulations which survived thermodynamic stability tests, were taken for dispersibility test (Table 3). Thermodynamic stability studies differentiate those nanoemulsion formulations from those of kinetically stable formulations which undergo phase separation. The formulations which pass the thermodynamic stability studies are those formulations which contain adequate amounts of ScoS concentration required for nanoemulsion formulation, and which decreases the energy required for nanoemulsion formation. This decreased energy contributes to the stability of nanoemulsion. The nanoemulsion formulations on entering the GI tract undergo infinite dilution leading to phase separation of the formulation due to poor aqueous solubility of the drug. Formulations which passed the dispersibility studies were certain to remain as nanoemulsion upon dispersion in the aqueous environment of the GIT. For oral nanoemulsion the process of dilution by the GI fluids will result in the gradual desorption of the surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration.

Formulations which passed thermodynamic stability tests and dispersibility test were subjected to globule size analysis, refractive index determination, viscosity determination and *in vitro* release studies (Figure 6).

A. Characterization studies

Optimized formulations selected from phase diagram at a difference of 5% w/w of oil having least SCoS concentration that passed dispersibility test were selected, named as formulation A, B, C, D and E. They were subjected to *in vitro* characterization studies (Table 4).

The globule size increases with increase in concentration of oil in formulation and decreases with increase in the concentration of SCoS. The mean globule size of the formulation A containing 25% oil was 90 nm while as formulation, B containing 30% oil was 254.3 nm and formulation D with 15% oil was 40 nm. Since the globule size of the droplets was much smaller than that of the blood capillaries (400 nm), there are minimal chances of capillary blockage during transport of the droplets. Thus higher circulation time of the droplets after *in vivo* application is also favoured. The Polydispersity Index (PDI) of formulation A and C was 0.287 and 0.398 suggesting uniformity in the globule size of the formulation but in the case of formulation B and D the PDI was 0.723 and 0.910 so these formulations were dropped from further studies.

Zeta potential of a formulation relates to its colloidal stability. It indicates the degree of repulsion between adjacent and similarly charged particles in the dispersion. For molecules that are small enough a high zeta potential confers stability and resists aggregation. The zeta potential of the formulations A and C were -19.0 and -22, indicates that the formulations are stable.

The viscosity of the optimized formulations was determined. It was observed that viscosity of all the formulations is less than 31 cp. Formulation A and C has the minimum viscosity 22.3 cp and 19.2 cP, while B has highest viscosity of 30.1 cp perhaps because of its higher oil content. Lower viscosity is an ideal characteristic of the o/w nanoemulsion.

Conductivity of the optimized formulations was found in range of 451-522.3 µS/cm. From the viscosity and the electro-conductivity study it is concluded that the system is of o/w type.

The refractive index of the developed system was similar to the refractive index of the water (1.333). In addition, the developed system showed percent transmittance >97%. The observed transparency of the system is due to the fact that the maximum size of the droplets of the dispersed phase is not larger than 1/4th of the wavelength of visible light. Thus, NE scatters little light and was therefore transparent or translucent.

From the above analysis the formulation A was selected for drug incorporation and *in vitro* and *in vivo* studies.

B. Scanning electron microscopy (SEM):

The scanning electron microscopic study reveals the external morphology of the nanoparticles and from Figure 7 it was evident that maximum nanoparticles were nearly spherical in shape.

Calibration curves: Calibration curves for olanzapine were

developed in 0.1 N HCl (pH 1.6), Biorelevant media at λ_{max} 254 and equation for the graphs are y= 96377x with regression coefficient R^2=0.9927 and y=98951x with regression coefficient R^2=0.9949 respectively.

Bioanalytical calibration curve for olanzapine was developed in plasma whose equation is y=0.0007x with regression coefficient R^2=0.997.

In vitro drug release

Comparative *in vitro* dissolution in 0.1n hcl and biorelevant media: *In vitro* dissolution studies were performed in biorelevant media and 0.1 N HCl. The fasted state conditions were used for dissolution studies since IVIVC is developed in fasted state. Comparative dissolution studies were performed to investigate the drug dissolution from SNEDDS, marketed tablet formulation containing same quantity of drug (2.5 mg). *In vitro* dissolution studies showed that Olanzapine is rapidly released ≥ 98 % from the optimized SNEDDS into the biorelevant media while it was ≥ 96% in 0.1 N HCl. Out of this 81% of the drug released during first hour of the study in both the media. In contrast, the marketed formulation has shown a release of ≥ 74%. This is because of the small globule size, and eventually higher surface area in case of SNEDDS, which permits faster rate of drug release.

A. *In vivo* bioavailability studies

In vivo bioavailability studies were performed to quantify SNEDDS formulation after oral administration and to compare the bioavailability of SNEDDS with that of pure drug suspension and marketed formulation. From the table 7 it can be inferred that the SNEDDS formulation showed maximum plasma concentration of 82.79 ng/ml by the end of first hour while the pure drug suspension and marketed formulations showed only 54.93 ng/ml and 59.12 ng/ml release by that time respectively. The pure drug suspension and marketed formulations took 2 hours to reach maximum plasma concentration of 58.31 ng/ml and 62.78 ng/ml respectively. From the above analysis it is evident that the SNEDDS formulation showed improved rate of drug release compared to the conventional marketed formulations. The SNEDDS showed maximum drug concentration in half of the time taken by the pure drug suspension and the marketed formulation.

B. Pharmacokinetic parameters

Pharmacokinetic parameters were calculated from the *in vivo* release of Olanzapine in rabbits for pure drug suspension, SNEDDS, and marketed tablet were calculated using excel sheet and absorption rate constant by Wagner-Nelson method.

Since the rate limiting step in the absorption of Olanzapine is dissolution from the formulation and the results from the study reveals that the dispersion of the drug (since the drug is completely dissolved in the oil phase) into the aqueous gastrointestinal environment is the rate limiting step in case of SNEDDS and plays a major role for absorption. It can be explained that, following oral administration, SNEDDS disperse spontaneously to form a nanoemulsion in the GI fluid where the active components are present in a solubilized form, and the small droplet size provides a large surface area for drug absorption. Such an ultra-fine dispersion of the oil will afford rapid and extensive absorption. In addition high concentration of surfactant in SNEDDS may increase permeability of the oil across the cell membrane, and lymphatic transport through the transcellular pathway.

The SNEDDS formulation not only reached maximum plasma concentration levels within half the time required by the pure drug suspension and the marketed formulation but also showed faster rate of elimination from plasma. The relative bioavailability of the marketed formulation and SNEDDS was 1.3 and 1.6 when compared to pure drug suspension. The fraction bioavailability of SNEDDS when compared to marketed formulation was found to be 1.2. From the above discussion it was evident that SNEDDS showed improved bioavailability when compared to the pure drug suspension and the marketed formulation.

In vitro and *in vivo* correlation

In vitro and *in vivo* correlation has been developed between the fraction of drug absorbed in bioavailability studies conducted in New Zealand white rabbits and the fraction of drug dissolved *in vitro* in biorelevant media and 0.1 N HCl.

Level A correlation was developed with a correlation factor R^2=0.99 in both the media. It represents a point to point relationship. From this we can assume that both the biorelevant dissolution media and 0.1N HCl mimics the *in vivo* absorption kinetics of the formulation. Thus 0.1 N HCl can be used *in vitro* instead of *in vivo* studies which are economical and time saving. Hence *in vivo* bioavailability studies can be waived.

Conclusion

A nanoemulsion formulation SNEDDS of Olanzapine was formulated with optimized *in vitro* characteristics and improved *in vitro* and *in vivo* dissolution of the drug. The composition of biorelevant media represents that of physiological gastro-intestinal media which the formulation encounters after oral administration. From the comparative *in vitro* dissolution studies performed both in 0.1 N HCl and biorelevant media; it was observed that the release behavior in both the media were similar. Hence 0.1 N HCl could be used as a media of choice instead of biorelevant media. From the comparative *in vitro* dissolution studies performed it can be concluded the SNEDDS formulation showed better bioavailability and drug disposition characteristics than pure drug suspension and the conventional marketed formulation. From the *in vivo* bioavailability studies performed, it can be concluded that the SNEDDS are with enhanced rate of drug release *in vivo* compared to pure drug suspension and the marketed formulation. IVIVC has been developed for *in vitro* dissolution of drug in both 0.1 N HCl and biorelevant media and *in vivo* bioavailability of drug in rabbit plasma. The IVIVC studies resulted in Level A correlation which suggests that the *in vitro* dissolution of drug in 0.1 N HCl could be used as a surrogate method for *in vivo* bioavailability of drug.

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