

# Studies on Physico-Chemical and Pharmacokinetic Properties of Olanzapine through Nanosuspension

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## Abstract

The increasing frequency of poorly water soluble new chemical entities exhibiting therapeutic activity is of major concern to the pharmaceutical industry. Olanzapine is an atypical antipsychotic used orally in treatment of Schizophrenia. Also, it has poor aqueous solubility (BCS Class II drug) whose oral bioavailability has been reported as only 40%. In this study olanzapine nanosuspension was prepared using Solvent diffusion followed by sonication technique. The nanosuspension was characterized for particle size distribution, poly dispersity index, zeta potential, crystallinity study (DSC), invitro dissolution release profile and pharmacokinetic studies. Average size of the nanoparticles in F6 was 122.2nm. Saturation solubility of optimized batch of nanosuspension and the plain drug were found to be 2851.3±6.3 µg/ml and 251.3 ±6.1 µg/ml, respectively. *In vitro* cumulative release from the nanosuspension was 83.54% at 45 min when compared to pure drug 22.91% and freeze dried nanosuspension 92.67%. Pharmacokinetic studies in rats revealed that AUC<sub>(0-∞)</sub> was increased and clearance was decreased when Olanzapine nanosuspensions were administered orally compared with that of Olanzapine suspension which in turn 2 folds increased bioavailability. The enhanced relative bioavailability by the formulation might be attributed to oral bioavailability can be attributed to the adhesiveness of the drug nanosuspension, increased surface area (due to reduction in particle size), increased saturation solubility, leading to an increased concentration gradient between the gastrointestinal tract lumen and blood, and increased dissolution velocity. This enhancement in bioavailability will lead to a subsequent reduction in drug dose, rendering the therapy cost-effective and obliterating any undue drug dumping in the body. Thus, Nanosuspension seems to be a promising approach for bioavailability enhancement because of the simple method of its preparation and its universal applicability.

**Keywords:** Olanzapine, Poor solubility, *in-vitro* dissolution, Nanosuspensions, Oral delivery, Bioavailability

## INTRODUCTION

It is a well known fact that majority of new chemical entities (approx. 60%) of the drugs coming directly from synthesis are poorly soluble. Consequently many of these substances have bioavailability problems after oral administration [1]. From this, there is a definite need for smart technological formulation approaches to make such poorly soluble drugs bioavailable. A Nano suspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. A pharmaceutical Nano suspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral and topical use or parenteral and pulmonary administration. [2].

Nano suspensions differ from nanoparticles. Nanoparticles are commonly polymeric colloidal carriers of drugs whereas solid lipid nanoparticles are lipidic carriers of drugs. In nano suspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size < 10 µm) is related to an increase in the surface area and consequently the dissolution velocity. Nano sized particles can increase solution velocity and saturation solubility because of the vapour pressure effect[4]. In addition, the diffusional distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient.

The increases in surface area and concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Furthermore, the saturation solubility is increased as well.

Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. Dissolution experiments can be performed to quantify the increase in the saturation solubility of a drug when formulated into a nano suspension [5].

The stability of the particles obtained in the nano suspension is attributed to their uniform particle size which is created by various manufacturing processes. The absence of particles with large differences in their size in nano suspensions prevents the existence of different saturation solubility's and concentration gradients, consequently preventing the Oswald ripening effect [6]. Ostwald ripening is responsible for crystal growth and subsequently formation of micro particles. It is caused by a difference in dissolution pressure/saturation solubility between small and large particles. Molecules diffuse from the higher concentration area around small particles which have higher saturation solubility to an area around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles. Nano suspension technology has been developed as a promising candidate for efficient delivery of hydrophobic drugs [7]. This technology is applied to poorly soluble drugs that are insoluble in both water and oils. Preparation of nano suspensions were reported to be a more cost effective and technically more simple alternative, particularly for poorly soluble drugs and yield a physically more stable product than liposomes; conventional colloidal drug carriers [8].

Olanzapine [OL] is a psychotropic agent that belongs to the thienobenzodiazepine class and is indicated for acute and maintenance treatment of schizophrenia [9]. It has been approved by FDA because it causes less extrapyramidal effects than do conventional antipsychotics. It is practically insoluble in water and suffers from low bioavailability (40%) and also wide variety of side effects are associated with current dosage forms of the drug. It is eliminated extensively by hepatic first pass metabolism [10]. In the recent years many research groups have shown interest to utilize drug loaded nanosuspension drug delivery using technique like solvent diffusion [11]. In view of this, we have developed and characterised Olanzapine nanosuspensions for improved oral delivery.

## MATERIALS AND METHOD

### Materials

Olanzapine was a kind gift from Indo-Swift pvt Ltd (Chandigarh, India), Tween 80 and Pluronic F-68 were purchased from Sisco (India). Dialysis Bag was purchased from Hi-Media (India). Acetonitrile (HPLC Grade) and Water (HPLC Grade) were purchased from Merck (India). All other chemicals and reagents were of analytical

### Preparation of Nano Suspension

The preparation of nano suspension was done by solvent diffusion method. Drug was weighed and dissolved in 10 ml of Organic Solvent (Acetone) required quantity of surfactants such as Tween 80 and Pluronic F-68 were dissolved in 20ml of water by using Mechanical Stirrer. The drug solution was added drop by drop to the above solution and was stirred (3000rpm) about 30mins and was subjected to sonication (at 80 amplitude for 15min). Cryoprotectant Mannitol was added before subjecting to freeze drying. The prepared nano suspension was subjected to lyophilisation and the dried product was collected and characterized. The composition of nanosuspension is given in Table no 1. [12]

### Evaluation of Nanosuspensions

#### Particle Size and zeta potential:

Particle size and zeta potential of the formulations were measured by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). All size and zeta potential measurements were carried out at 25°C using disposable polystyrene cells and disposable plain folded capillary zeta cells, respectively, after appropriate dilution with original dispersion preparation medium.

#### Polydispersity index:

Polydispersity was determined according to the equation,

$$\text{Polydispersity} = \frac{D(0.9) - D(0.1)}{D(0.5)}$$

Where, D (0.9) corresponds to particle size immediately above 90% of the sample. D (0.5) corresponds to particle size immediately above 50% of the sample. D (0.1) corresponds to particle size immediately above 10% of the sample [13].

#### Saturation solubility

Approximately 1.5 ml of prepared nanosuspension was filled in 2 ml centrifugation tube and centrifuged after 24 h, using sigma centrifuge at 25000 rpm for 30 mins. Concentration of olanzapine in the supernatant was

measured spectrophotometrically using UV-Visible spectrophotometer (Shimadzu) at 258 nm after suitable dilution with PH 6.8 Phosphate buffer using the same solvent as blank. Saturation solubility of plain drug was also measured in the similar manner[14].

#### Differential Scanning Calorimetry (DSC)

DSC analysis was performed using DSC Q200 (TA Instruments, USA). A heating rate of 10°C/min was employed at a range of 20-210°C. Analysis was performed under nitrogen purge (50ml/min). A standard aluminium sample pans were used. About 5mg sample was taken for analysis. An empty pan was used as reference. DSC thermograms were recorded for Olanzapine (OL), and OL loaded nanosuspensions.

#### External Morphological Study

External morphology of nanoparticles was determined using Scanning Electron Microscopy (SEM). The surface morphology of solid lipid nanoparticles was observed after sputter coating the samples with gold using a scanning electron microscope (FEI QUANTA 200 SEM/EDAX) at 30 kV as accelerating voltage. The images were captured under different 12,000 x magnification and recorded.

#### Invitro release studies

The releases of Olanzapine from all the three formulations were studied under sink conditions. Formulations which showed higher drug content and entrapment efficiency, saturation solubility were evaluated for *in vitro* release. 5ml of formulations equivalent to 1mg were put in dialysis bags (MWCO 12000, HiMedia). The dialysis bags were placed in 50mL of dissolution medium ( Phosphate buffer pH 6.8 ) and stirred under magnetic stirring at 37°C. Aliquots of the dissolution medium were withdrawn at each time interval and the same volume of fresh dissolution medium was added to maintain a constant volume. Samples withdrawn from buffer were analyzed for Olanzapine content spectrophotometrically at 258 nm against solvent blank[15].

#### Stability studies

Stability studies for nanosuspension and lyophilized nanosuspension were conducted at two different storage conditions, viz., room temperature and refrigerated conditions (2–8°C) for 3 months. Each one batch of nanosuspension and lyophilized nanosuspension were used for each storage condition. At periodic time intervals, the samples were withdrawn and analyzed for particle size and drug content

#### Pharmacokinetic studies

Albino Wistar rats (Male and Female) weighing 200±20g were used for oral bioavailability studies. All animal experiments were approved by CPCSEA and IAEC, J.S.S. College of Pharmacy, Ootacamund, India. All the rats were fasted for 12h before the experiment but had free access to water. OL-SUSP and formulations were administered to 6 rats in each group (Male: Female; 1:1) by oral feeding tube at the dose of 9mg/kg of Olanzapine. Blood samples were collected via the caudal vein at 0,0.17,0.33, 0.5, 0.67, 0.83, 1, 2,3,4 and 6 hours after administration separately. Blood samples were placed into tubes containing 0.3ml of anticoagulant solution and centrifuged immediately. After centrifugation, the plasma obtained was stored at -20°C until further analysis.

### Quantification of plasma concentration

Olanzapine plasma concentration was determined by HPLC analysis. A 200 $\mu$ l plasma sample was placed into a centrifuge tube and 200 $\mu$ l of 10% perchloric acid was added and shaken vigorously for 30s at room temperature. After centrifugation at 400 rpm for 15 min, the supernatant was separated and analyzed. Calibration curves were prepared by linear regression analysis of the plot of the peak area against concentration of olanzapine. The concentration of plasma samples was determined from the area of chromatographic peak using the calibration curve.

### HPLC and analytical method

The HPLC system consisted of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array detector (SPD-M20 A; Shimadzu, Japan) and a 20  $\mu$ L loop (Rheodyne). A C<sub>18</sub> reverse-phase column (Phenomenex Gemini C<sub>18</sub>, 250 x 4.6mm i. d., 5 $\mu$ ) was utilized for drug separation, using acetonitrile-25mM Potassium dihydrogen orthophosphate pH 6.5 (50:50,v/v) as mobile phase. The flow rate and UV wavelength were 1.0 ml min<sup>-1</sup> and 254nm, respectively.

### Data Analysis

Peak concentration (C<sub>max</sub>) and time of peak concentration (T<sub>max</sub>) were obtained directly from the individual plasma-concentration time profiles. The area under the concentration-time curve from time zero to time t (AUC<sub>0-t</sub>) and AUMC was calculated using the trapezoidal method. The area under the curve (AUC) determines the bioavailability of the drug for the given same dose in the formulation. The area under the total plasma concentration-time curve from time zero to infinity was calculated by: (AUC<sub>0-∞</sub>) = AUC<sub>0-t</sub> + C<sub>t</sub>/K<sub>e</sub> where, C<sub>t</sub> is the Olanzapine concentration observed at last time, and K<sub>e</sub> is the apparent elimination rate constant obtained from the terminal slope of the plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The relative bioavailability (F<sub>r</sub>) at the same dose was calculated as: F<sub>r</sub> = AUC<sub>SLN, 0-t</sub> / AUC<sub>SUSP, 0-t</sub>. The mean residence time (MRT) was estimated from MRT = AUMC<sub>0-∞</sub> / AUC<sub>0-∞</sub>.

The data obtained from the release rate and pharmacokinetic parameters were analyzed by *t*-test. Statistically significant differences were assumed when *p*<0.05. All the values expressed are their mean  $\pm$  S.D.

## RESULTS AND DISCUSSION

### Zeta Potential

Among the different formulations, batch (F6) has shown the Zeta Potential of -25.1mV which shows the good stability. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. Particles in this formulation are small, with a high zeta potential will confer stability, i.e. the solution or dispersion will resist aggregation. Pluronic F-68, a non-ionic surfactant is used as a stabilizer, which provides steric stabilization. So, negative zeta potential is attributed

to drug nanocrystals. In general, zeta potential value of  $\pm$ 20 mV is sufficient for stability of nanosuspension stabilized by steric stabilizer Poloxamer 407.[16]

### Particle size and Polydispersity Index (PI)

The polydispersity index (PI) is the measure of size distribution of the nanoparticles. PI was measured using Malvern zetasizer. PI values range from 0.000 to 1.000 i.e. monodisperse to very broad particle size distribution. The batch (F2) containing Tween 80(0.25mL) and Pluronic F-68(250 mg) as surfactant lowest PI of 0.116 and avg. particle size of 122.2nm shown in figure 1 . A decrease in particle size with increase in the Poloxamer 407 concentrations and stirring time was observed. OL nanosuspension based final formulation is intended for oral route. According to Moschwitz the typical particle size of a nanosuspension is around 200–1000 nm [17]. The desirable attributes for a nanosuspension are maximum saturation solubility and particles in the nanometric size range. All the prepared batches do exhibit the size in the nanometer range. PI values of all the formulations indicate that particle size distribution was narrow or unimodal.

### Saturation solubility

Among the formulations, batch number F6 was found to have maximum saturation solubility of 2851.3 $\pm$  6.3  $\mu$ g/ml and hence it was considered to be an optimized batch for further studies. Saturation solubility of optimized batch of nanosuspension and the plain drug were found to be 2851.3 $\pm$  6.3  $\mu$ g/ml and 251.3  $\pm$  6.1  $\mu$ g/ml, respectively shown in Table no 2. Thus, there is 11.31-fold increase in the saturation solubility of OL when it is formulated as a nanosuspension. This great increase in saturation solubility is attributed to dual role played by the presence of surfactant and smaller particle size, especially below 1–2  $\mu$ m and subsequent increase in surface area.. As OL belongs to BCS class II drug i.e. low solubility high permeability, an increase in the solubility is expected to improve the absorption and thus bioavailability[18,19].

### Differential Scanning Calorimetry (DSC)

DSC is a tool to investigate the melting and recrystallization behaviour of crystalline material. Fig. 2 shows DSC curves of olanzapine and nanosuspension. The DSC curve of olanzapine showed a melting endotherm at 194.75°C. The thermograms of nanosuspension showed the melting endotherm peak of olanzapine 142.24 °C This suggests that olanzapine was not in crystalline state but is in polymorphic state. This melting point depression might be due to small particle size (nanometer range), the high specific area, and the presence of surfactant. This melting point depression can be attributed to Kelvin effect [20].

### External Morphological Studies

The External morphological studies using scanning electron microscope (SEM) revealed that maximum nanoparticles were nearly spherical in shape (Fig. 3). The result showed that mannitol was the most effective cryoprotectant in terms of preventing nanoparticles from aggregation. The nanoparticle size observed by SEM correlated well with the particle size measured by particle size analyzer[21].

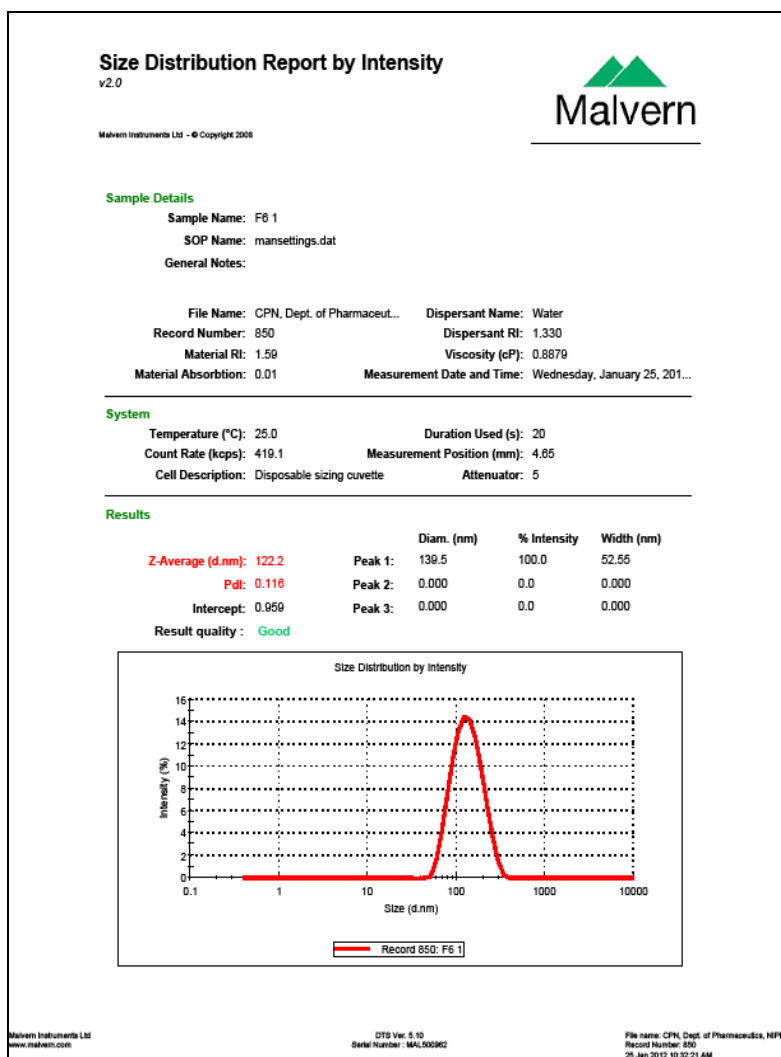


Figure.1 Particle size distribution of nanosuspension

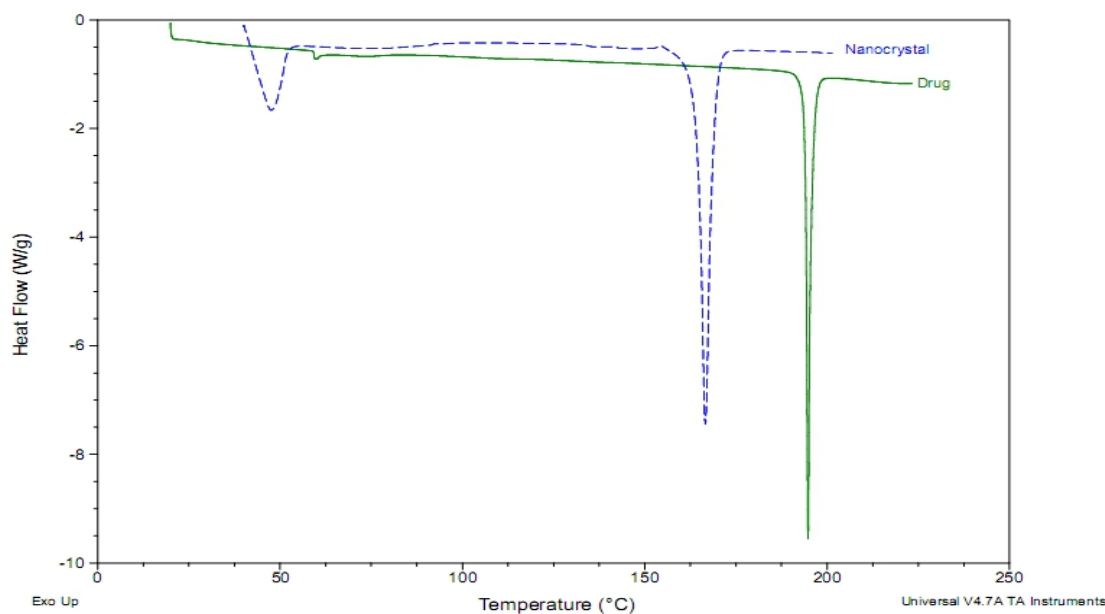


Figure.2 Scanning calorimeter curves of OL and OL- loaded nanosuspension.

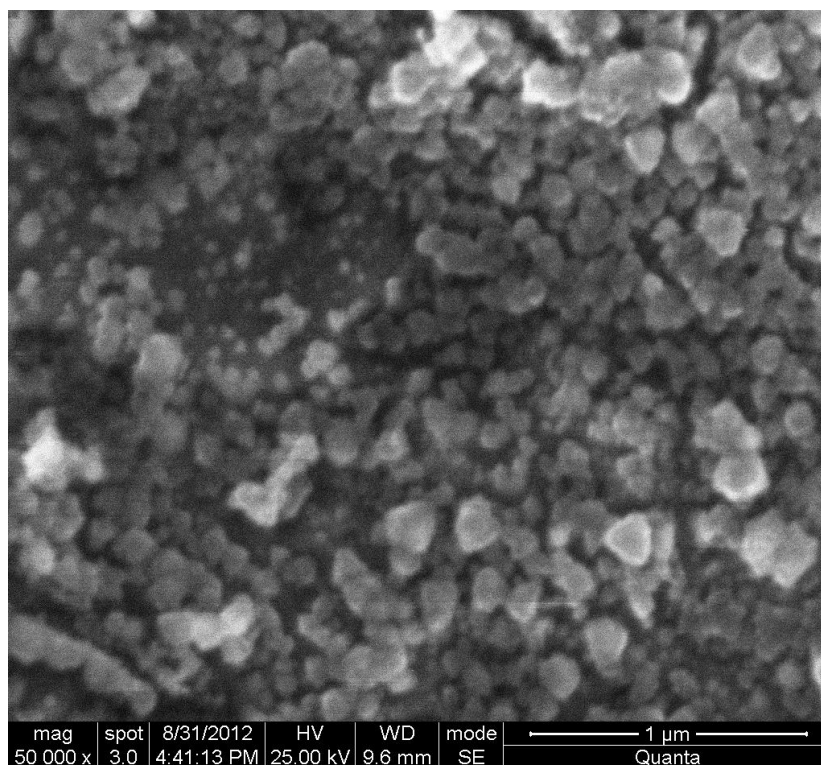


Figure.3 Scanning electron micrograph (SEM) of nanosuspension

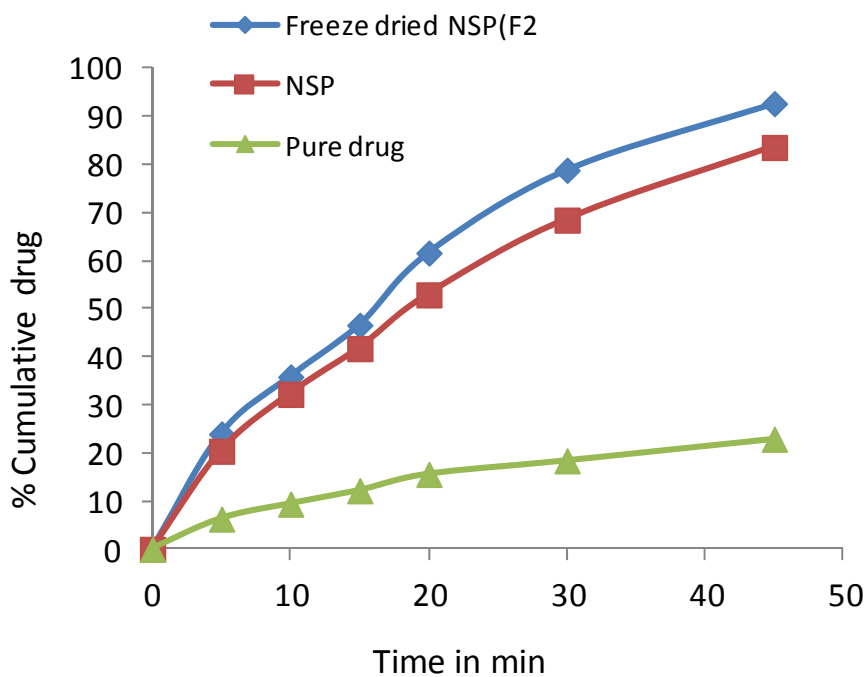


Figure.4 Comparative In vitro release profile in phosphate buffer 6.8

**Table 1** Composition of drug loaded batches

Formulation Code	Drug (mg)	Tween80 (ml)	Poloxamer 407 (mg)
NSP-1	5	0.25	250
NSP-2	10	0.25	250
NSP-3	5	0.25	500
NSP-4	10	0.25	500
NSP-5	5	0.5	250
NSP-6	10	0.5	250
NSP-7	5	0.5	500
NSP-8	10	0.5	500

**Table 2** saturation Solubility profile of olanzapine in 0.1 N HCL (mean± S.D., n = 3)

Drug Loaded Batch	Solubility
F6	2851.3 ± 6.1 mg/mL
F8	1892.7 ± 2.8 µg/mL

**Table 3** Effect of storage time (at 25°C) on particle size and drug content of nanosuspension formulations (mean± S.D., n = 3)

Nanosuspension	Particle size (nm)	Drug content (%)
0 day	277.5±5.2	87.29±0.82
1 month	279.4±2.4	85.45±2.14
2 month	282.2±3.1	84.12±1.46
3 month	284.4±4.8	89.41±1.98

**Table 4** Comparative Pharmacokinetic parameters of olanzapine pure drug suspension and nanosuspension after oral administration (mean ± S.D.) (\* p < 0.05)

Parameter	OL-SUSP	OL- Nanosuspension
C <sub>max</sub> (µg/mL)	2.91 ± 0.04	4.76 ± 0.13*
t <sub>max</sub> (h)	2.18 ± 0.08	1.05±0.12*
t <sub>1/2</sub> (h)	1.76± 0.04	2.0±0.10*
AUC <sub>0→t</sub> (µg h/mL)	5.75 ± 0.37	10.75 ± 0.76*
AUC <sub>0→∞</sub> (µg h/mL)	7.40 ± 0.71	13.44±0.61*
K <sub>el</sub> (1/h)	0.39 ± 0.01	0.34±0.61*
F <sub>r</sub>	1	2

### Invitro release studies

The main attribute of a nanosuspension is the increased dissolution rate resulting from the high specific surface area of the particles, dissolution of the powders was performed as a pharmaceutical evaluation. Dissolution profile Figure 4 shows the dissolution profile of the plain drug and lyophilized nanosuspension. In case of lyophilized nanosuspension, more than 70% drug dissolved within 30 mins and about 92% dissolved within 45 mins, while the plain drug showed only 6% dissolution at the end of 5 mins and 22% after 45 mins. There is a significant difference between the dissolution rates of the plain drug and the nanosuspension. This drastic increase in the dissolution rate is due to the increased surface area of the drug due to smaller size and also due to presence of the surfactant. For

lyophilized nanopowder, the combination of excellent disintegration properties of Mannitol and the high surface area of the OL nanoparticles improved the dissolution profile, showing complete dissolution within minutes. According to Noyes–Whitney equation, an increase in saturation solubility and decrease in particle size lead to an increased dissolution rate. It is reported that the saturation solubility increases with decreasing particle size. However, this effect is only pronounced for particle below approximately 2 µm, especially below 1 µm[22,23]. So formulation of poorly water-soluble drugs as nanometer sized drug particles has a dramatic effect on dissolution rate, drug solubility and consequently bioavailability. The bioavailability of OL nanosuspension is truly dissolution rate limited, so particle size reduction can significantly improve the performance of the drug.

### Stability studies

In the case of nanosuspension stored at room temperature, the particle size increased from 122.2 to 134.8 nm in 90 days shown in Table no 3. However, under refrigerated storage conditions, there was a nominal increase from 122.2 to 126.2 nm indicating better stability under these conditions. The results showed that temperature has an influence on aggregation of nanoparticles and at room temperature, aggregation was higher compared to refrigerator condition for liquid nanosuspension. Refrigerated condition has no significant effect on particle size whereas room temperature condition has more detrimental effect. The conclusion is that higher temperature results in increase in particle size. The effect is more significant in liquid nanosuspension as compared to dry formulation. The increase in the particle size at room temperature is thought to be due to the aggregation of the particles. Another reason may be the Ostwald ripening resulting from fluctuations in room temperature. It can be observed that there is no significant change in the drug content of the formulation whether liquid or dry, under any of the two storage conditions viz. room temperature or refrigerated conditions. Thus, both the liquid and lyophilized nanosuspensions are chemically stable at both the storage conditions. However, lyophilization and storage under refrigerated conditions is recommended for better physical stability.

### Pharmacokinetic studies

Pharmacokinetic parameters of olanzapine after oral administration are shown in Table 4. The C<sub>max</sub> value of olanzapine in OL nanosuspension was significant (p<0.05) than that observed with OL-SUSP shown in Table no 4. The AUC<sub>0→t</sub> value of olanzapine after oral administration of OL nanosuspension was 2 fold higher than those obtained with OL-SUSP. Possible explanation for significantly higher (p<0.05) AUC than OL-SUSP is due to the great increase in saturation solubility is attributed by the presence of surfactant and smaller particle size, especially below 1–2 µm and subsequent increase in surface area from OL nanosuspension compared to OL-SUSP which lead to an increased in the solubility and improved the absorption and thus bioavailability.

The influence of stabilizer can be degradation accelerating (e.g. sodium cholate, lecithin) or a degradation slowing down effect due to steric stabilization (e.g. Poloxamers).

Thus, intermediated degradation time can be obtained by mixing the degradation accelerating and hindering stabilizer [24]. In present study, Tween 80 and poloxamer 188 were chosen as stabilizers. A number of studies have reported an improvement in oral absorption of poorly soluble drugs by co-administration of various P-glycoprotein inhibitors [25]. Poloxamer 188 might moderately inhibit the P-glycoprotein efflux system, leading to improved oral absorption of olanzapine [26]. The use of nanosuspension opens up new perspectives for the formulation of poorly soluble drugs.

### CONCLUSIONS

In this study, Formulation of nanosuspensions of Olanzapine was successfully carried out by solvent diffusion technique and characterized. The physico-chemical characterization shown that crystalline Olanzapine was converted to polymorphic form (DSC Study) which evidenced by enhanced dissolution rate in comparisons of formulation with Olanzapine (pure drug). The formulated nano suspension has shown 2 fold increased relative bioavailability when compared to the pure drug. The increased drug dissolution rate may have a significant impact in absorption which in turn the improved oral bioavailability of Olanzapine. Thus, this delivery system may prefer to improve the dissolution of poorly soluble drugs like olanzapine thus enhanced oral bioavailability.

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