Chapter 10

Formulation optimization and characterization of Olanzapine nanosuspension

10.1 INTRODUCTION

Olanzapine is an atypical antipsychotic which is widely used in the management of acute and maintenance treatment of schizophrenia and related psychotic disorders. Recent studies have revealed that OL penetration into brain is greater in Transgenic Abcb1a P-glycoprotein deficient mice than FVB1 (wild-type) animals (Wang JS et al., 2004). This result obviously shows that the entry of OL into the brain is dependant on the transmembrane energy-dependent efflux transporter P-glycoprotein (Pgp). Pgp is highly polymorphic and its expression in the endothelial cells of the blood–brain barrier (BBB) limits substrate access to the central nervous system (CNS), thereby influencing the therapeutic consequences of psychoactive drugs (Thompson JS et al., 2000). P-gp plays a protective role in major organs by limiting cellular uptake of xenobiotics by excreting these compounds into bile, urine, the intestinal lumen, and limiting accumulation in brain. This activity of P-gp as a drug efflux pump plays an important role in absorption, distribution, and elimination of various xenobiotics

Various approaches like administration of P-gp inhibitors, use of hyper osmotic agents to artificially open the BBB, increasing the lipophilicity of the xenobiotic, utilization of an endogenous celluar transport system that is present in the BBB, e.g transferrin receptor on the surface of brain capillary endothelial cells has been utilized for the transport of iron into the brain (Friden PM., 1995), direct implantion of biodegradable polymeric devices into the brain by intra cerebral injection have been followed by researchers in order to increase the brain delivery of drugs by passing the P-gp efflux system at the BBB. The potential of colloidal drug carriers like nanoparticles, liposomes to enhance drug delivery to brain is being investigated by various researchers around the globe (Schroder U et al., 1998). It is interesting that the nanoparticle-mediated drug transport to the brain depends on the coating of particles. Inspite of showing promise as a potential drug delivery system, not many reports are available on nanosuspensions as a drug delivery system to the brain.

Wang and co workers determined P-gp inhibition of some commonly used excipients using an integrated high-throughput process (Wang SW et al., 2004). From the list of excipients reported by Wang SW and co workers, we have selected poloxamer 407 and d – α - Tocopherol Polyethylene Glycol Succinate 1000 for preparation of OL nanosuspension. It was hypothesized that the above excipients can inhibit the P-gp drug efflux pump present at the BBB and enhances the brain delivery of olanzapine.

In the present chapter, the nanosuspensions of OL were prepared and characterized. The nanosuspensions were also subjected to stability evaluation at different storage conditions. The pharmacokinetics and biodistribution studies were performed for the nanosuspensions using radiolabeling technique to assess their potential for brain delivery of OL (Chapter 12).

10.2 MATERIALS AND METHODS

Materials

Olanzapine (OL) was received as a gift sample from Cadila Healthcare, India. Poloxamer 407 was received as a free gift sample from BASF, USA. d – α - Tocopherol Polyethylene Glycol Succinate 1000 (TPGS) was obtained as a free gift sample from Eastman Chemicals, USA. Zirconium oxide beads of diameter 0.4-.0.7 mm were purchased from S.D Fine Chemicals, India. All other chemicals used in the study were of analytical grade. Water used in all the studies was distilled and filtered through 0.22 μm nylon filter before use.

Methods

Preparation and optimization of OL Nanosuspensions

OL nanosuspensions were prepared by pearl milling technique (Verhoff F et al., 2003). Briefly, 100 mg of poloxamer 407 was dissolved in 10 ml of distilled water in a 20 ml glass vial. 400 mg of Zirconium oxide beads of size 0.4- 0.7mm was added (40%w/v of the batch size). 300 mg of OL was added slowly to the above milling chamber. Milling was initiated by magnetic stirring at 5000 rpm for 4 hours. The nanosuspension was obtained in a powder form by lyophilization using 1:3 sucrose (with respect to total solid content) as cryoprotectant (POL NS). TPGS stabilized OL nanosuspension was prepared in the same way as above by replacing poloxamer 407 with TPGS (TOL NS).

The effect of concentration of stabilizer (poloxamer 407 / TPGS), volume of milling media and milling time on the initial size of the nanosuspension was studied. Effect of milling time was determined by removing aliquots from the milling vessel at various time intervals and measuring the particle size. The effect of the volume of milling media on the mean particle size was studied by varying the media between 30% to 50%w/v of the batch size. Effect of stabilizer (poloxamer/ TPGS) on the mean particle size of the nanosuspensions formed was studied by varying the concentration between 0.5% to 1.0%w/v.

Assay of OL in the OL nanosuspensions

Both POL NS and TOL NS were weighed and dissolved in 0.1 HCL and the solutions were analyzed using a UV – Visible spectrophotometer (Hitachi U2000, Japan) at the λ_{max} of 258 nm.

Characterization

Determination of saturation solubility

The saturation solubility of OL and both POL NS and TOL NS in PB pH 7.4 was determined by adding excess OL in PB pH 7.4 and mechanical shaking for 24 h to attain dissolution equilibrium. After 24 h, the dispersion was centrifuged at 15,000 rpm for 20 minutes in a cooling centrifuge (Sigma, Osterode, Germany) to sediment the undissolved drug. The absorbance of the supernatant was determined at 276nm using a UV–Visible spectrophotometer (Hitachi U2000, Japan).

Measurement of size and zeta potential of OL nanosuspensions

Size and zeta potential of the nanosuspensions were measured by photon correlation spectroscopy (PCS) using Malvern Zetasizer ZS (Malvern Instruments, UK). Samples were diluted appropriately with distilled water pre-saturated with OL (in order to avoid reduction in particle size during dilution) for the measurements.

Differential Scanning Calorimetry (DSC)

DSC analysis was carried out using a Differential scanning calorimeter (DSC-60, Shimadzu, Japan) at a heating rate of 10°C per minute in the range of 30°C to 250°C under inert nitrogen atmosphere at a flow rate of 80ml/min. DSC thermograms were recorded for OL, POL NS and TOL NS.

X- ray diffraction studies (XRD)

Powder X-ray diffraction patterns were obtained using an X-ray diffractometer (Philips PW 1710) with Cu Kα radiation generated at 30 mA and 40 kV. The source of X - ray was copper anode with a wavelength of 1.54060 Å. The XRD patterns were recorded for OL, POL NS and TOL NS.

Transmission Electron Microscopy (TEM) studies

TEM studies were performed in Morgani transmission electron microscope. Nanoparticles were dispersed in de ionized water and one drop of the reconstituted nanoparticles was incubated on 200 – mesh carbon coated copper grid. The copper grid was fixed into sample holder and placed in vacuum chamber of the transmission electron microscope and observed under low vacuum (10^{-3} torr)

Determination of in vitro steric stability by electrolyte induced flocculation test

The electrolyte induced flocculation test was performed for the olanzapine nanodispersions stabilized with 1.0% poloxamer 407 (POL NS) and 1.0% TPGS (TOL NS). The effect of poloxamer 407 and TPGS on the ability of the nanoparticle to resist electrolyte induced flocculation was investigated by this test. Sodium sulphate solutions ranging from 0 M to 1.5 M were prepared in 16.7 %w/v sucrose solution (Subramanian N et al., 2003). An appropriate volume of nanodispersion was made up to 5 ml using the sodium sulphate solutions of varying concentrations (0 M,0.3 M,0.6 M,0.9 M,1.2 M and 1.5 M) to obtain a final concentration of 1mg/ml OL. The absorbance of the resulting dispersions was measured within 5 min at 400 nm using a UV–Visible spectrophotometer (Shimadzu, Japan) against respective blank.

Stability studies

The effect of storage temperature was determined by conducting short-term stability studies for the POL NS and TOL NS dispersions for a period of three months. The particle size and zeta potential of the OL nanosuspension immediately after milling was measured. Then this batch was divided into three portions and stored in sealed transparent glass vials (USP type I) under different temperature conditions of 4°C (in a refrigerator), 30°C (ambient room temperature) and 40°C (temperature regulated oven) in a black colored box. Samples after 7 days, 15 days, 1 month and 2 months were subjected to particle size and zeta potential measurements. Stability studies were also carried out for the lyophilized powder of both the nanosuspensions at the same temperature mentioned above for a period of 6 months. The mean particle size, time for reconstitution and the difference in the invitro release pattern from the initial in vitro release pattern was studied.

In vitro release studies

In vitro release of OL from the nanosuspensions (both POL NS and TOL NS) was determined in PB pH 7.4. The OL nanosuspension was placed in a dialysis bag (cutoff molecular weight of 12000 Daltons, Himedia, India) and sealed at both ends. The dialysis bag was then dipped into the receptor compartment containing the dissolution medium. The release of OL from an aqueous OL dispersion (as control) through the dialysis bag was also studied. The dissolution media was continuously stirred at 100 rpm and maintained at 37 ± 2°C. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh dissolution medium. All the dissolution samples were analyzed spectrophotometrically at 276 nm against a solvent blank for drug content. All experiments were repeated thrice and the average values were taken.

10.3 RESULTS AND DISCUSSION

Influence of milling time

The initial particle size of bulk OL was 17.06 ± 0.44μm prior to pearl milling. Pearl milling (using 40%w/v zirconium beads of 0.4mm-0.7mm) for 4 h resulted in smaller sized particles with mean particle diameter of 0.295 ± 0.02μm (uniformity value 0.202) and 0.310 ± 0.02μm (uniformity value 0.181) for POL NS and TOL NS respectively. Further milling beyond 4 h did not result in significant reduction in particle size. Particle size after 6 hours pearl milling resulted in particles with mean particle diameter of 0.291± 0.01μm for POL NS and 0.303 ± 0.02μm with TOL NS. The effect of volume of beads and milling time on the mean particle size diameter of the POL NS is tabulated in table 10.1 and 10.2 respectively. The effect of volume of beads and milling time on the mean particle size diameter of the TOL NS is tabulated in table 10.3 and 10.4 respectively

Wet milling is associated with possible contamination of the product with residues of milling media arising out of erosion of the milling material. Hence, the contamination level of milling media, zirconium in the final nanosuspension dispersion was determined by atomic absorption spectroscopy. The residual amount of zirconium in the final nanosuspension was detected to be as low as 12 ppm. Keck and co workers (Keck CM and Muller RH., 2006) reported that the contamination levels of the milling media in the final product generally depends upon the hardness of the drug and the milling material and also the time required for milling time which usually ranges from hours or up to several days.

Short term stability of both the nanosuspensions in the dispersion state revealed that they were unstable. Hence their recovery in the powder form is essential to improve the shelf life. Lyophilization of the dispersions was carried out in the presence of sucrose as cryoprotectant (1:3 with respect to total solid content). Nanosuspensions lyophilized with less than the above mentioned sucrose ratio yielded powders which were hygroscopic in nature in the case of POL NS and waxy powders in case of TOL NS. Lyophilization of nanosuspensions has been already employed by Peters and co workers to increase the stability of clofazimine nanosuspension for intravenous use (Peters K et al., 2000).

Volume of beads (%w/v)	**Mean Particle Size diameter ± S.D (nm)**	**Uniformity value**
25	441 ± 6.61	0.621
30	376 ± 5.42	0.514
35	332 ± 4.78	0.331
40	***295 ± 6.02***	***0.202***
45	289 ± 4.98	0.221
50	290 ± 5.12	0.210

TABLE 10.1: Effect of volume of beads on the particle size of poloxamer 407 stabilized olanzapine nanosuspension (milling time: 4 hours; using 0.4-0.7mm zirconium beads)

Milling Time (hours)	Mean Particle Size Diameter (nm)	Uniformity value
Initial	17060 ± 481.26	1.544
0.5	8463 ± 354.51	1.022
1	1054 ± 20.80	0.895
2	566 ± 5.91	0.399
4	***295 ± 6.02***	***0.202***
6	291 ± 4.10	0.210

TABLE 10.2: Effect of milling time on the particle size of poloxamer 407 stabilized olanzapine nanosuspension (Volume of beads – 40%w/v; using 0.4mm–0.7mm zirconium beads)

Volume of beads (%w/v)	Mean Particle Size diameter ± S.D (nm)	Uniformity value
25	725 ± 4.22	0.524
30	462 ± 4.07	0.334
35	360 ± 3.47	0.249
40	***310 ± 5.24***	***0.181***
45	319 ± 4.42	0.202
50	314 ± 4.22	0.221

TABLE 10.3: Effect of volume of beads on the particle size of TPGS stabilized olanzapine nanosuspension (milling time: 4 hours; using 0.4-0.7mm zirconium beads)

Milling Time (hours)	Mean Particle Size Diameter (nm)	Uniformity value
Initial	17060 ± 481.26	1.544
0.5	8009 ± 400.11	1.223
1	1123 ± 38.18	1.014
2	611 ± 7.17	0.650
4	***310 ± 5.24***	***0.181***
6	303 ± 4.45	0.200

TABLE 10.4: Effect of milling time on the particle size of TPGS stabilized olanzapine nanosuspension (Volume of beads – 40%w/v; using 0.4mm–0.7mm zirconium beads)

Differential Scanning Calorimetry

The crystalline structure of the nanosuspensions can be determined by differential scanning calorimetry (DSC). This is especially important when a drug exists in different polymorphic forms. The DSC curves for the bulk OL, POL NS and TOL NS are shown in figure 10.1. The DSC curve of bulk OL (Figure 10.1) showed a melting endotherm of the drug at 197.99°C. This peak was found absent in the thermograms of POL NS and TOL NS (Figure 10.1). There was a shift in the melting endotherm of the drug in both the nanosuspension formulations (at 208.74°C for POL NS and 214.06°C for TOL NS). Further this melting endotherm was not observed as a distinct, sharp transition. This indicates the presence of OL in an amorphous state after formulating into nanosuspension. This may be attributed to increased lattice defects in the drug crystal, which in turn reflects reduced degree of crystallinity as a result of pearl milling. Crytallinity index was determined by the enthalpy of the bulk drug as 100% (Saupe A et al., 2005). The crytallinity index was found to be 35.07% and 64.63% for POL NS and TOL NS respectively. The DSC parameters calculated from the DSC thermograms are shown in table 10.5.

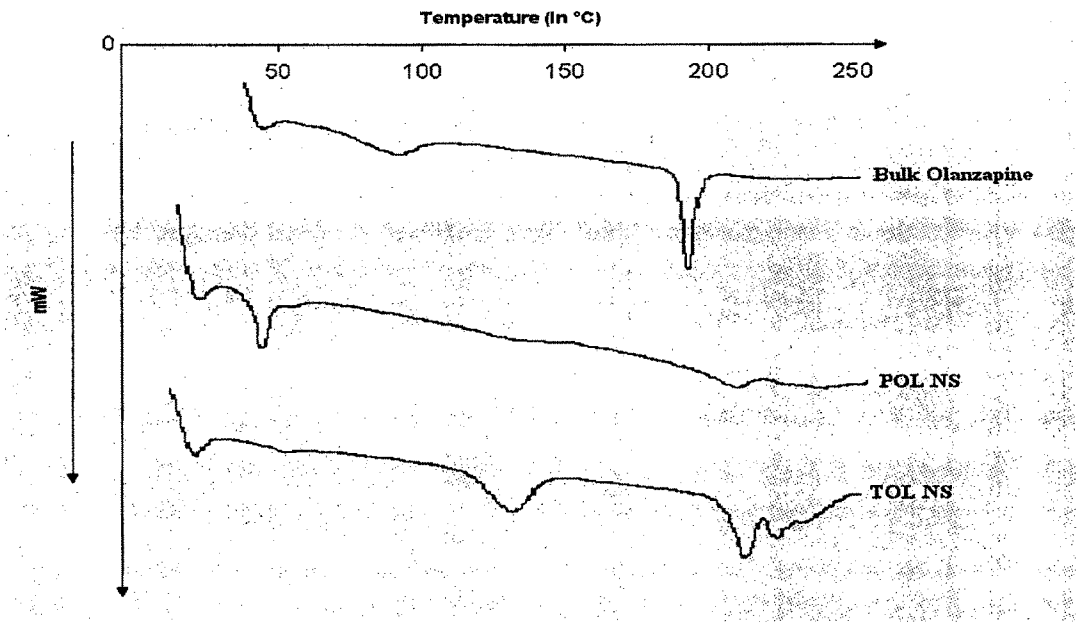


FIGURE 10.1: Differential Scanning Calorimetry thermograms of Bulk OL, POL NS and TOL NS

DSC Parameters	Bulk Olanzapine	Olanzapine Nanosuspension	
		POL NS	TOL NS
Enthalpy (J/g)	118.67	41.54	76.71
Onset (°C)	191.81	198.87	205.62
Peak (°C)	197.99	210.14	214.41
Crytallinity Index	100.00%	35.07%	64.63%

TABLE 10.5: Differential scanning calorimetry data of Bulk olanzapine and both the nanosuspension

Powder X- ray diffraction studies (PXRD)

Crystal diffraction software tools are widely used to simulate PXRD patterns as reference standards for individual crystal forms (such as polymorph, solvates, and salts). The XRD pattern of bulk OL showed a total of 32 peaks while the XRD patterns of POL NS and TOL NS showed a total of 11 and 16 peaks respectively. The standard peak in OL was found at a diffraction angle (°2θ) of 8.535 with a *d* value of 10.3517 Å. In the XRD pattern of POL NS, the standard peak was found at a diffraction angle of 23.215 with a *d* value of 3.8284 Å and in TOL NS, the standard peak was found at a diffraction angle of 16.180 with a *d* value of 5.4737 Å. These values indicate the possible change in crystal form of OL in both POL NS and POL NS. The crystallinity index of olanzapine after conversion into nanosuspension was calculated by considering the intensity of the principle peak obtained with bulk olanzapine as 100%.

Formulation	**Peak intensity at angle 8.535 °2θ [counts]**	**Crystallinity index (%)**
Bulk Olanzapine	1011	100.00
POL NS	139	13.75
TOL NS	274	27.10

TABLE 10.6: X - ray diffraction data of bulk olanzapine and both olanzapine nanosuspensions

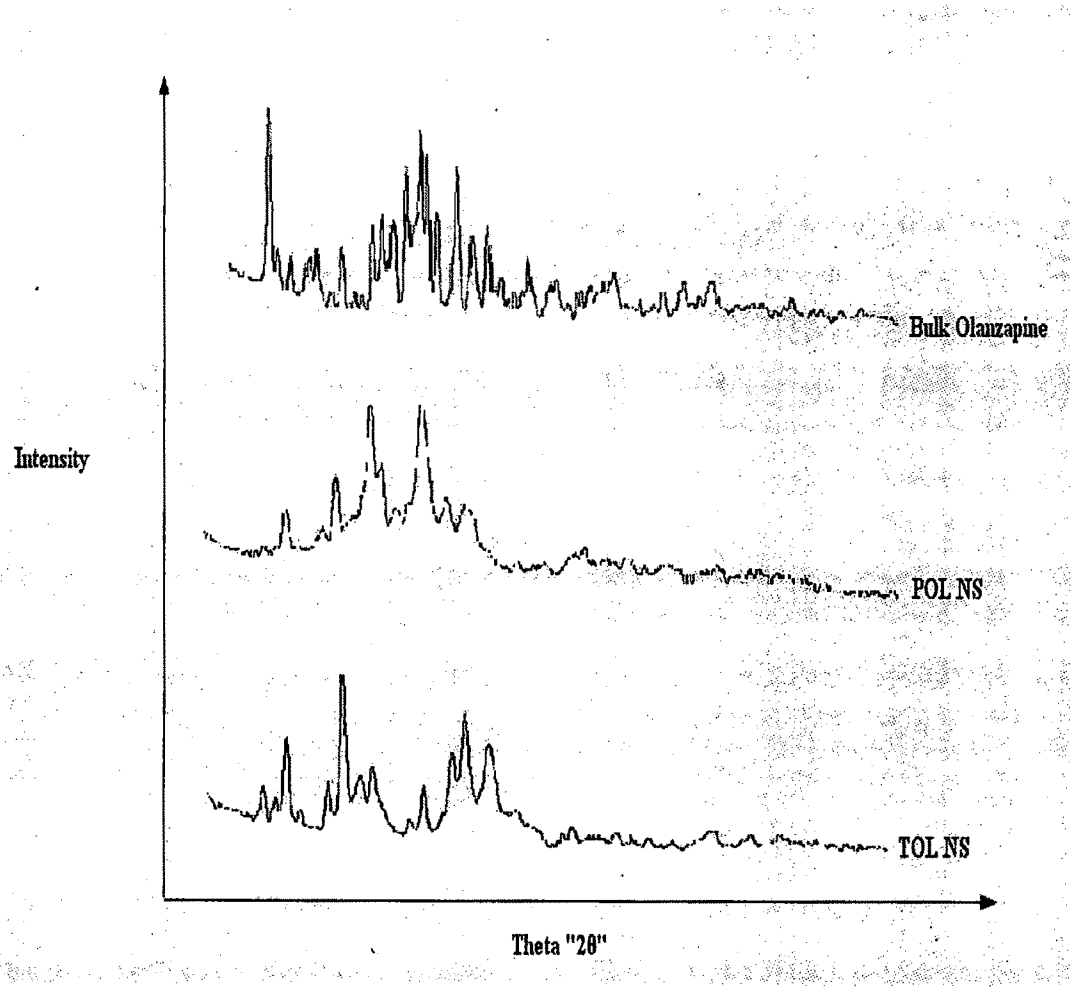
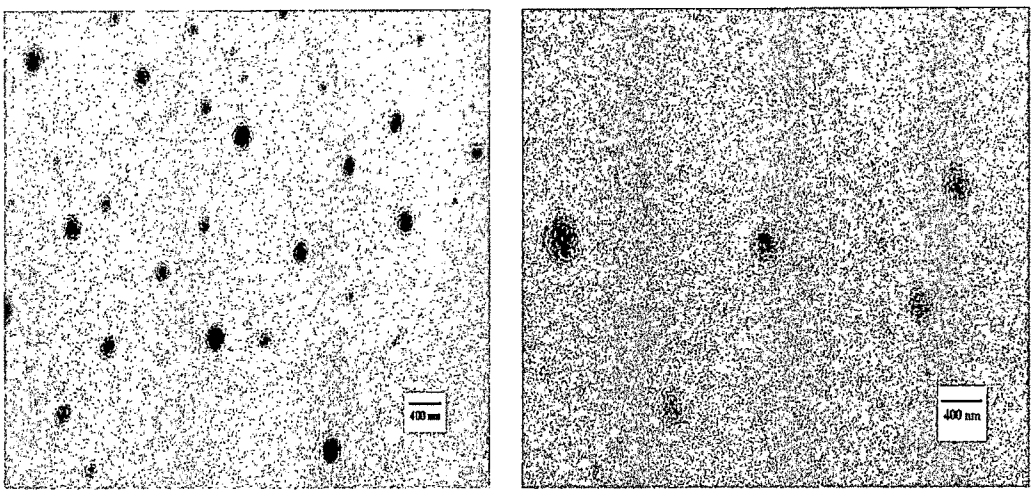


FIGURE 10.2: X – ray diffraction pattern of Bulk OL, POL NS and TOL NS



A B

FIGURE 10.3: Transmission electron micrograph of POL NS (A) and TOL NS (B)

Determination of in vitro steric stability by electrolyte flocculation test

The effect of poloxamer 407 and TPGS on the ability of the nanoparticles to oppose electrolyte induced flocculation was investigated by this test. Coating the particulate systems with hydrophilic surfactants provide steric stability by rendering a hydrophilic surface, which in turn reduces the binding of serum opsonins and also cells of the reticuloendothelial system. Addition of electrolyte compresses the electrical double layer around the particle. This results in flocculation of the particles with a corresponding increase in optical turbidity of the particle dispersion which can be measured by the absorbance of the dispersion at 400nm. The scattering of the sample increased by the inverse 4^{th} power of the wavelength of the incident light, and hence authors used a lower wavelength (400nm) was used for the measurements

In the present investigation, POL NS showed a gradual increase in the flocculation as the concentration of electrolyte (sodium sulphate) was increased beyond 0.6M. TOL NS showed a gradual increase in the absorbance as the concentration of electrolyte was increased beyond 0.3M. Beyond this concentration, a sharp increase in the flocculation was observed. The results are given in figure 10.4.

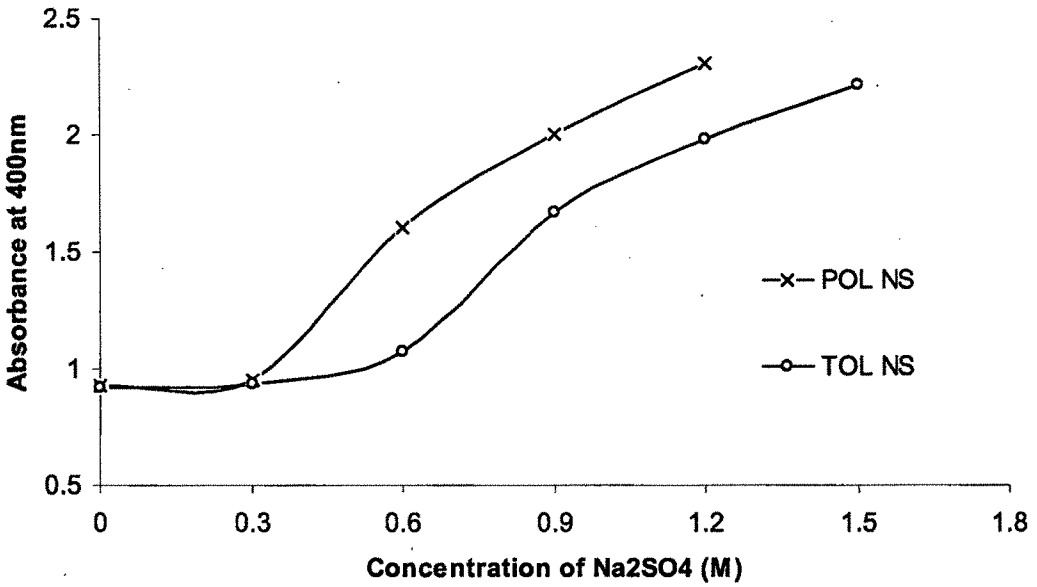


FIGURE 10.4: Steric stabilization effect of the olanzapine nanosuspension.

Saturation solubility and in vitro release studies

The saturation solubility of bulk OL in PB pH 7.4 was found to be 8.87 ± 0.42μg/ml at room temperature (around 27-30°C). The aqueous solubility of OL increased considerably after formulating as nanosuspension. The saturation solubility of POL NS and TOL NS was 31.82 ± 1.09μg/ml and 27.96 ± 0.91μg/ml respectively. The increase in solubility in case of POL NS and TOL NS was almost 4-folds higher than the plain OL. Plain OL as dispersion form in PB pH 7.4 showed 97.63 ± 0.59% dissolution in about 120 min whereas POL NS showed 99.02 ± 0.81% dissolution in 60 min and TOL NS showed 98.26 ± 1.16% in 60 min. The release profiles clearly indicated the faster dissolution rate of OL in nanosuspension forms. The t_{25} (time taken for 25% release of OL), t_{50} (time taken for 50% release of OL) and t_{90} (time taken for 90% release of OL) values for the plain olanzapine dispersion and the two nanosuspension formulations are shown in table 10.7.

Saturation solubility is dependent on the interfacial tension σ, i.e. the interfacial energy G (G = σ * A) where A is the surface area of the particle, according to Ostwald–Freundlich equation.

Muller and co workers attributed differences in interfacial energy as a reason for the differences in saturation solubility of polymorphic forms (Muller RH et al., 1998). The higher surface energy (by virtue of their higher surface area) of nanosuspensions is responsible for higher saturation solubility and subsequent dissolution. Increase in the saturation solubility can also be explained by the possible creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. This in turn increases the exposure of the inner hydrophobic surfaces of the drug crystal to the aqueous dispersion medium.

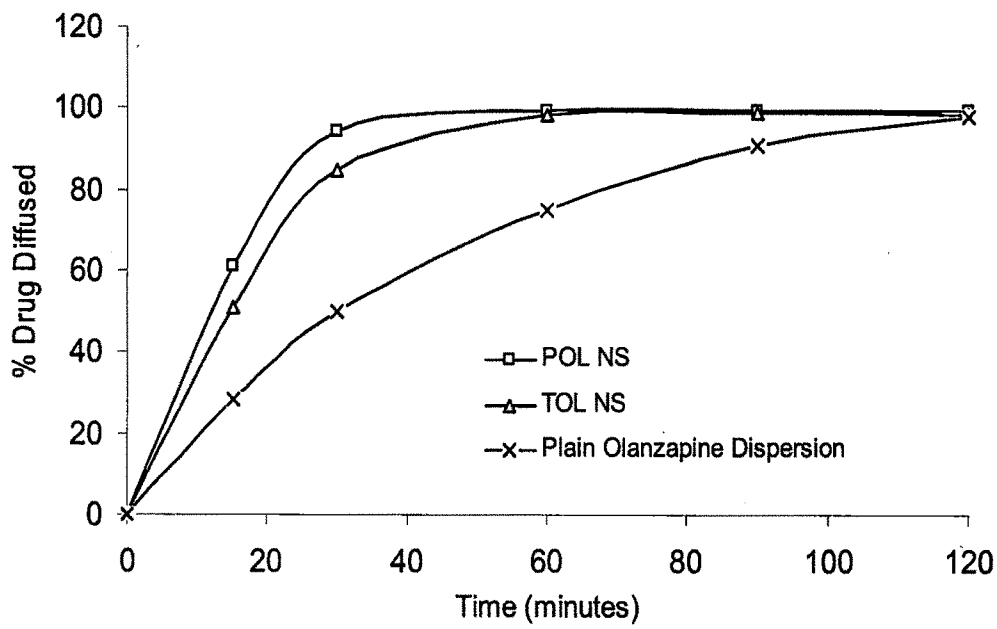


FIGURE 10.5: In vitro diffusion of OL from the OL solution and OL nanosuspension in phosphate buffer pH 7.4

Parameter	Time in minutes		
	Olanzapine dispersion	Poloxamer 407 stabilized olanzapine nanosuspension	TPGS stabilized olanzapine nanosuspension
t_{25}	13.24 ± 1.31	5.22 ± 0.93	8.01 ± 0.88
t_{50}	31.55 ± 1.12	12.75 ± 1.50	15.11 ± 1.07
t_{90}	71.53 ± 1.73	27.34 ± 1.01	35.40 ± 0.82

Table 10.7: Comparative t_{25} ± S.D (time taken for 25% olanzapine to be released) t_{50} values (time taken for 50% olanzapine to be released) and t_{90} (time taken for 90% olanzapine to be released) of olanzapine dispersion and both the nanosuspension formulations

Stability studies

Effect of temperature on particle size

The particle size of both the nanosuspensions was found to increase with the increasing time duration of storage, as a result of particle aggregation. Mean particle diameter of the POL NS stored at 40°C increased from 295 ± 5.91nm to 983 ± 8.81nm in 3 months, whereas, for samples stored at 4°C and 30°C, it increased only to 409 ± 4.22nm and 779 ±5.98nm respectively (Figure 10.6). In case of TOL NS samples stored at 40°C, mean particle diameter of the particles increased from 310 ± 3.92nm to 1196 ± 8.36nm, whereas, for samples stored at 4°C and 30°C, it increased to 551 ± 4.55nm and 909 ± 7.70nm respectively (Figure 10.7).

Another observation seen in all the stability samples was the increase in the sedimentation velocity of the particles. However, this sedimentation was not harmful, as simple shaking again resulted in a homogenous nanodispersion. Similar phenomenon was also observed by Huettenrauch and co workers (Huettenrauch R and Moeller U., 1983) during their study on the milling of sulfathiazole.

As the storage temperature increase, there is a increase in input energy which leads to the destabilization of the nanosuspension dispersions. The input energy increase the kinetic energy

of the particles which in turn leads to increased inter-particle collision and finally particle aggregation. Increase in particle size can also be explained by Ostwald ripening principle, which is mainly due to the differences in saturation solubility above the surface of differently sized crystals and sufficiently high changes in solubility with temperature changes.

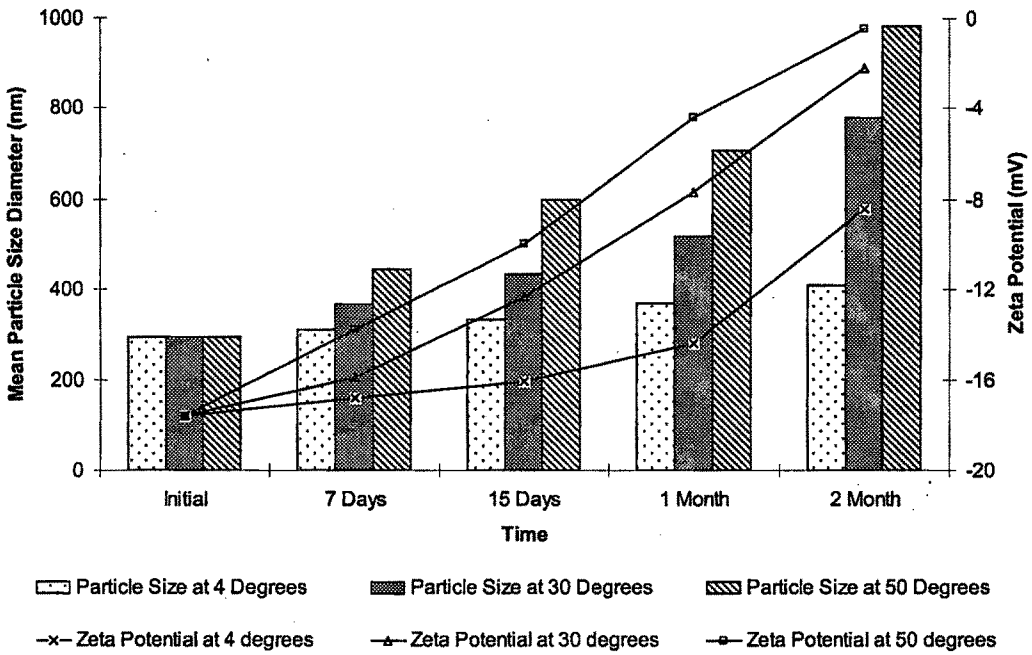


FIGURE 10.6: Mean particle Diameter and zeta potential of POL NS dispersion stored at different temperatures in dark condition.

Effect of storage temperature on zeta potential

The zeta potential of both the nanosuspensions was found to increase with the increasing time duration of storage. Zeta potential of the POL NS stored at 40°C increased from -17.6 mV to -0.424 mV in 3 months, whereas, for samples stored at 4°C and 30°C, it increased only to -8.43 mV and -5.12mV respectively (Figure 10.6). In case of TOL NS samples stored at 40°C, mean particle diameter of the particles increased from -21.6 mV to +1.34 mV, whereas, for samples stored at 4°C and 30°C, it increased to -6.92 mV and -3.31 mV respectively (Figure 10.7).

Surface stabilization can be accomplished by either charged surfactants (anionic or cationic) or non–ionic polymers. Both poloxamer 407 and TPGS are non – ionic in nature. Non-ionic stabilizers attach their hydrophobic domains at multiple sites on the particle surface. The probability that these hydrophobic moieties will detach from the particle surface at room temperature is very low, consequently providing a strong surface affinity (Alexandridis P and Hatton TA., 1995). A system is considered stable if the electrostatic repulsion dominates the attractive van der Waals forces. When the kinetic energy of the particle is high enough to overcome the barrier of electrostatic repulsion, they undergo collision. Increase in temperature usually leads to increase in kinetic energy of a system, which in combination with a reduction in zeta potential leads to the aggregation of nanosuspension (Freitas C and Muller RH., 1998).

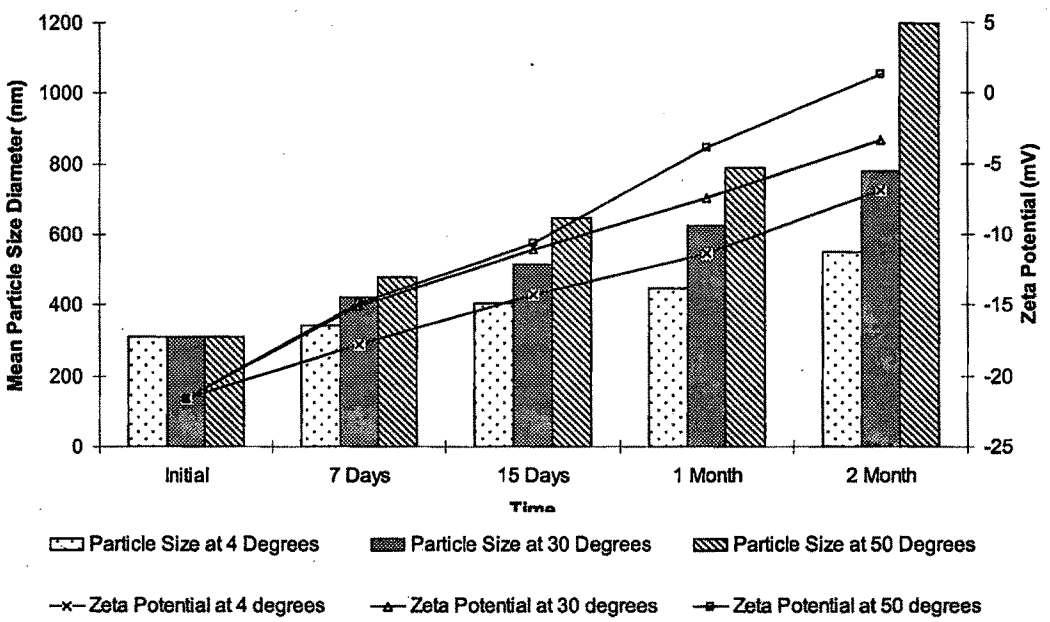


FIGURE 10.7: Mean particle Diameter and zeta potential of TOL NS dispersion stored at different temperatures in dark condition.

In vitro release studies were carried out for the both the lyophilized POL NS and TOL NS after 1, 2, 3 and 6 months at three temperature conditions (4°C, 30°C and 40°C in dark condition) and compared with the initial in vitro release profile. To study the variability in dissolution

data, the value of difference factor (f_1) and similarity factor (f_2) were calculated using the Moore and Flanner equation (Saranadasa H and Krishnamoorthy K., 2005). Moore and co workers used a simple model independent approach which utilizes a difference factor (f_1) and similarity factor (f_2) to compare dissolution profiles (Moore JW and Flanner HH., 1996). The difference factor (f_1) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves. For curves to be considered similar, f_1 values should be close to 0, and f_2 values should be close to 100. Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test and reference products. The mean particle size diameter, time for reconstitution and f_1 and f_2 values for the samples stored in the different temperature conditions are shown in table 10.7 and 10.8.

Time (Months)	Samples Stored at 4°C				Samples Stored at 30°C				Samples Stored at 40°C			
	Mean Particle Size Diameter (nm ± S.D)	Time for reconstitution (seconds ± S.D)	In vitro release		Mean Particle Size Diameter (nm ± S.D)	Time for reconstitution (seconds ± S.D)	In vitro release		Mean Particle Size Diameter (nm ± S.D)	Time for reconstitution (seconds ± S.D)	In vitro release	
			F1	F2			F1	F2			F1	F2
1	318 ± 3.67	12 ± 2.14	0.6	98.0	330 ± 3.46	15 ± 1.31	1.2	95.0	345 ± 3.45	16 ± 1.69	1.0	95.1
2	329 ± 4.02	12 ± 2.07	0.8	97.1	348 ± 4.26	15 ± 2.10	1.3	92.3	364 ± 2.41	16 ± 1.75	1.6	91.4
3	343 ± 3.54	13 ± 2.34	0.8	97.4	365 ± 2.99	15 ± 2.08	1.8	90.3	379 ± 4.62	18 ± 2.30	2.1	87.4
6	360 ± 3.74	13 ± 2.04	1.2	96.1	380 ± 3.31	16 ± 1.97	2.0	88.1	392 ± 3.35	18 ± 2.46	3.5	81.8

Table 10.7: Stability data for poloxamer 407 stabilized olanzapine nanosuspension (in powder form)

Initial Particle Size Diameter 313 ± 2.99 nm

Initial time for reconstitution 11 ± 2.15 seconds

Time (Months)	Samples Stored at 4°C				Samples Stored at 30°C				Samples Stored at 40°C			
	Mean Particle Size Diameter (nm ± S.D)	Time for reconstitution (seconds ± S.D)	In vitro release		Mean Particle Size Diameter (nm ± S.D)	Time for reconstitution (seconds ± S.D)	In vitro release		Mean Particle Size Diameter (nm ± S.D)	Time for reconstitution (seconds ± S.D)	In vitro release	
			F1	F2			F1	F2			F1	F2
1	322 ± 2.45	15 ± 3.35	0.8	97.0	334 ± 2.56	19 ± 2.31	0.9	96.0	338 ± 1.95	20 ± 1.56	1.2	93.9
2	330 ± 3.14	18 ± 2.28	0.9	96.8	348 ± 2.44	20 ± 2.06	1.1	94.3	350 ± 2.54	22 ± 1.94	1.7	90.0
3	336 ± 2.78	17 ± 2.98	0.9	96.4	359 ± 2.61	20 ± 1.88	1.5	92.2	366 ± 3.00	23 ± 2.02	2.4	84.7
6	342 ± 2.49	20 ± 2.54	1.1	95.0	370 ± 3.03	22 ± 1.40	1.8	89.8	381 ± 3.38	23 ± 1.66	4.0	77.8

Table 10.8: Stability data for TPGS stabilized olanzapine nanosuspension (in powder form)

Initial Particle Size Diameter 319 ± 3.17 nm

Initial time for reconstitution 14 ± 2.19 seconds

10.4 CONCLUSIONS

Pearl milling of OL for 4 hours in the presence using zirconium oxide beads (size 0.4- 0.7mm) yielded nanoparticles of smallest size in case of both poloxamer stabilized and TPGS nanosuspensions. Olanzapine after formulation into nanosuspension improved its saturation solubility and dissolution in vitro. The nanosuspension dispersions can be successfully converted into the powder form by either lyophilization to increase its shelf life. There was a change in the crystallinity of OL after conversion into nanosuspension state. Electrolyte flocculation test revealed that the nanosuspension formulations tended to agglomerate in electrolyte concentrations beyond 0.6M sodium sulphate in case of POL NS and 0.3M in case of TOL NS indicating possibility of higher steric stabilization.

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