Chapter 6

Formulation optimization and characterization of Ziprasidone loaded poly (lactide co -glycolide) microparticles

6.1 INTRODUCTION

Medication non-compliance is a major barrier to better health outcomes for people with schizophrenia. At least 50% of out patients with schizophrenia stop their medication within a year of hospital discharge and this non-compliance is a major risk factor for relapse which may turn out to be more severe and dangerous (Babiker IE., 1986). Most physicians feel that high rate of relapse; hospitalization and suicidal behavior associated with untreated schizophrenia indicate that continuous antipsychotic treatment is the most likely solution to successful therapy. Generally schizophrenic patients have difficulty to comply with their medication regimen because of the nature of the illness. Therefore, a long acting injectable medication that does not require the patient to take the medication daily might increase compliance and substantially improve patient symptoms (Nicola O'Connell, 2000).

Use of biodegradable polymers in prolonging drug release has attracted various researchers across the world (Elaiz RE et al., 2000; Wright JC et al., 2001; Brodbeck KJ et al, 1999). These novel drug delivery systems were well accepted by patients than the existing formulations and were also easier to administer to the patient. Among the various biodegradable polymers available, Poly lactide co-glycolide (PLGA) has found wide application in the preparation of drug delivery devices. PLGA is chemically inert in the conditions employed to prepare the microspheres and is available in a range of molecular weights and ratios of lactic and glycolic acid by which the release rate can be modified. Further, PLGA co polymers are non immunogenic, well tolerated and non toxic and are extensively used in the preparation of biodegradable sutures, staples, fixation rods, crews and clips (Bergsma EJ et al., 1993; Bezwada RS et al., 1995; Bostman O et al., 1991; Hashizoe M et al., 1994; Saitoh H et al., 1994; Suganuma J et al., 1993). The USFDA has approved many uses of these polymers. As drug carriers, biodegradable microspheres have the advantage of providing a large surface area for the drug release, easily administered and does not require removal after completion of drug release (Amsden B., 1999).

In spite of the need for a long acting antipsychotic formulation to combat non-compliance accompanied with Schizophrenia very few long acting antipsychotics are available in the market. Risperidone was the first atypical antipsychotic to be approved by the USFDA in a long acting PLGA microsphere based injectable formulation for the long-term treatment of Schizophrenia. ZB is an atypical antipsychotic belonging to the chlorooxyindole class aryl heterocyclic and is normally prescribed for the treatment of schizophrenia.

In the present chapter, ZB loaded PLGA 50:50 microspheres were prepared and characterized. The microspheres were also subjected to stability evaluation at different storage conditions. The pharmacokinetics studies were performed for the microspheres to assess their potential as an intramuscular depot formulation.

6.2 MATERIALS AND METHODS

Ziprasidone base (ZB) was received as a gift sample from Cadila Healthcare, Ahmedabad, India. Poly lactic-co-glycolide with monomer ratio of 50: 50 (PLGA 50:50) (PURASORB PDLG® inherent viscosity in chloroform, 25°C, 0.13 dL/g, (average molecular weight *M*w 15,000 Da) was obtained as a free gift sample from PURAC Biochem, Netherlands. Polyvinyl Alcohol (PVA) with molecular weight 72,000 was 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 of ZB loaded PLGA 50:50 microspheres

ZB loaded PLGA 50:50 microspheres (PLGA 50:50 MS) were prepared by simple o/w emulsification solvent evaporation method. (1,2). 100 mg of PLGA 50:50 was dissolved in 4 ml of chloroform using a vortex mixer (SPINX, Mumbai, India), 25 mg ZB (for theoretical drug loading 25%) was added to the above PLGA 50:50 in chloroform solution and dissolved using vortex mixer. This organic phase was slowly added to aqueous phase containing 1.0 % w/v polyvinyl alcohol (20 ml) under constant stirring (1500 rpm) (IKE WERKE, Germany). Solvent evaporation was carried out at room temperature under vacuum under continuous stirring for about 4 hours. After evaporation of chloroform, the microspheres were collected by centrifugation at about 3000 rpm for 10 minutes in a cooling centrifuge (Sigma, Osterode, Germany). The microspheres were washed using distilled water thrice to remove any adhered drug and stabilizer. The microspheres were then lyophilized using mannitol (1:2 ratio with respect to the total solid content) as a cryoprotectant. The lyophilized microspheres were stored in sealed USP type I glass vials at 4°C till further use. Various theoretical drug loadings (TDL) ranging from 5% to 30% were studied. The effect of stirring speed, stabilizer concentration on the microparticle size and drug loading efficiency were investigated to optimize the final formulation.

Selection of Oil phase: ZB distribution coefficient between oil and water

The selection of the suitable oil was selected by determining the distribution of ZB between the oil (dichloro methane, chloroform and ethyl acetate) and water was determined by the method already reported by Chen and co workers (Chen JL et al., 2002). The oil used and water were presaturated with each other before use at 25°C. Ziprasidone in oil solution (10mg/ ml) was prepared which was mixed with 5ml of water in a 10 ml glass vial and sealed. The samples were kept in a shaking incubator for 24 hours in a water bath maintained at 37°C. After 24 hours, the samples were collected by centrifugation at 2000 g for 30 min to separate the water and oil phase. ZB present in the oil layer was measured by separating the oil layer. The separated oil layer was evaporated to dryness and the residue was dissolved in tetra hydro furan and analyzed spectrophotometrically at 317nm for drug content in an UV – Visible spectrophotometer (Shimadzu 1610, Japan). The distribution coefficient of ZB in oil /water was calculated as following:

$$\text{Distribution coefficient} = C_{Zi} - C_{Ze} / 5\, C_{Ze}$$

where,

C_{Zi} = the initial concentration of ziprasidone in the oil layer

C_{Ze} = the concentration of ziprasidone in the oil layer after equilibrium for 24 hours.

Particle size analysis

The particle size analysis was performed by Laser scattering technique using a Malvern Hydro 2000SM particle size analyzer (Malvern Instruments, UK). The aqueous microsphere dispersion was added to the sample dispersion unit containing a stirrer and was stirred at about 1500 rpm. The laser obscuration was maintained between 10-20%. The analysis was performed thrice and the average values were taken.

Encapsulation efficiency

10mg of lyophilized microspheres were dissolved in 25 ml tetra hydro furan by vortexing in a cyclomixer (Remi Scientific Equipments, India). The solution was analyzed spectrophotometrically at 317nm for drug content in an UV – Visible spectrophotometer (Shimadzu 1610, Japan), after confirming the non-interference of the polymer (PLGA 50:50) in the absorbance region of ZB.

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. DSC thermograms were recorded for bulk ZB, bulk PLGA 50:50 and ZB loaded PLGA 50:50 MS.

X- ray diffraction studies (XRD)

X-ray diffraction was performed to study the degree of crystallinity and possible excipient interactions. X-ray patterns were obtained using a Philips PW 1710 with a Cu Kα radiation, θ to 2θ powder diffractometer set for an angle range of 5–60° 2θ. The samples scanned were bulk ZB and ZB loaded PLGA 50:50 MS.

Scanning electron microscopy

SEM photographs of the freshly lyophilized ZB loaded PLGA 50:50 MS and the ZB loaded PLGA 50:50 MS after 35 days dissolution were taken using a Scanning Electron Microscope (JSM 5610 LV SEM JEOL DATUM LTD., Japan). The microparticles were stuck on to a brass stub through double adhesive tape. The stub was fixed into sample holder and placed in vacuum chamber of scanning electron microscope and observed under low vacuum (10^{-3} torr)

Estimation of initial burst release and in vitro release studies

The in vitro burst release of ZB from the PLGA 50:50 MS was determined by the method reported by Costantino and co workers. (Costantino HR et al, 2000). Briefly, the lyophilized microspheres equivalent to 1mg ZB was placed in 20 ml pH 7.4 PB containing 0.1% tween 80. The microsphere suspensions were incubated at 37°C for 24h. The suspensions were centrifuged at 3000 rpm to sediment the microspheres, the supernatant was removed and analyzed spectrophotometrically at 317 nm. All experiments were repeated thrice and the average values were taken.

In vitro release of ZB was determined from the microspheres loaded with different quantities of ZB (5% to 30% theoretical drug loading) as reported by Schaefer and co workers (Schaefer MJ and Singh J., 2000) in phosphate buffer saline pH 7.4 containing 0.1% tween 80. A known amount of the lyophilized microspheres equivalent to 1mg of ZB were added to a 20 ml glass vial containing 10 ml of the dissolution media. The glass vial was closed with rubber stopper to prevent evaporation of the dissolution medium. The glass vials mounted on a rotating bottle

apparatus and rotated at 25 rpm. At regular time intervals (1, 3, 7, 14, 21, 28 and 35 days) samples were withdrawn. The sample was centrifuged at 3000 rpm to sediment the microspheres. The supernatant was removed and analyzed spectrophotometrically at 317 nm against the solvent blank. All the experiments were repeated thrice and the average values were taken.

Stability studies

Effect of Storage temperature

Short-term stability studies were conducted on the lyophilized ZB loaded PLGA 50:50 MS for a period of six months. Freshly lyophilized batch was divided into three portions and stored in transparent colorless glass vials under different temperature conditions of 4°C (in a refrigerator), 30°C (at room temperature) in dark condition by placing the samples in dark (black colored) box. Samples were withdrawn after 1 month, 2 months, 3 months and 6 months. The time required for reconstitution of the lyophilized powder and the particle size of the microspheres immediately after reconstitution was measured. Samples were also subjected to in vitro release tests. The difference in the initial in vitro release pattern and the in vitro pattern on storage was assessed by applying Moore and Flanner equation (Moore JW and Flanner HH., 1996). Photomicrographs of the microspheres were taken after 3 months to ascertain the possibility of particle agglomeration on storage (OLYMPUS DP-12, Japan).

6.3 RESULTS AND DISCUSSION

ZB distribution coefficient between oil and water

ZB is highly lipophilic with an aqueous solubility of 0.3μg/ml. Knowledge of the partition coefficient of the drug between the oil phase and the aqueous phase gives the formulator an idea regarding the amount of drug that may be lost by partition into the aqueous phase during preparation of microspheres.

Partition of ZB between three organic solvents (dichloro methane, chloroform and ethyl acetate) and water was determined. Ethyl acetate gave the least partition coefficient value of 2.39. Use of ethyl acetate in preparation of PLGA MS is not well reported maybe because of its high boiling point (76-78°C). Methylene chloride and chloroform are Class II solvents while Ethyl acetate is a Class IV solvent. Partition values obtained with chloroform and dichloro methane were 2.96 and 2.71 respectively. Microspheres prepared by o/w emulsion

solvent evaporation method using dichloro methane as the oil phase had a higher polydispersity index (0.496) than the microspheres prepared using chloroform (0.269). This observation can be explained by the fact that the rate of solvent removal can be controlled in a better way in the case of chloroform (because of its higher boiling point: 62-64°C) than dichloro methane (boiling point: 39-41°C). Faster rate of solvent removal associated with use of dichloro methane as the oil phase may have resulted in faster precipitation of the polymer particles in the external media leading to uneven particle size distribution. Chloroform in spite of its higher boiling point in comparison with dichloro methanewas selected for the preparation of PLGA microspheres.

Particle Size analysis

Mesens and co workers (Mesens J et al., 2004) after their work on Risperidone PLGA microsphere based depot system reported that microspheres with a particle size of 25μm to 180μm, preferably around 50μm is required for administration to the patient using hypodermic needles (Mesens J et al., 2004). This particle size range also results in maximum retention of the microparticles at the site of injection, hence providing a depot effect.

Influence of stabilizer concentration on microsphere formation

The influence of different concentrations of polyvinyl alcohol (PVA) ranging from 0.25% to 2.0%w/v on the size of microspheres was studied. When the concentration of PVA was increased from 0.25% to 2.0%, a decrease in size of all the microsphere formulations was observed. The batches prepared with 0.25% and 0.5% PVA lead to the microspheres with high value of polydispersity index indicating the instability of the emulsion formed and also insufficiency of the concentration of PVA to effectively stabilize the oil droplet (Polydispersity index value was 1.61 for 0.25% PVA and 1.15 for 0.5% PVA). The batches prepared with 0.75% to 2.0% PVA lead to microparticles with comparatively low uniformity values (polydispersity index was 0.541 for 1% PVA, 0.548 for 1.5% PVA and 0.404 for 2.0% PVA). The viscosity of PVA solutions increases with increase in PVA concentration, which may lead to the formation of a stable emulsion with a uniform droplet size.

The actual mechanism of stabilization of particle surface by PVA was believed to be by interpenetration of PVA and polymer molecules during microparticle formation (Sahoo SK et al., 2002). In case of PLGA, the hydrophobic segments of PVA penetrate into the organic

phase (chloroform) where they remain entrapped in the polymer matrix and the binding of PVA to the PLGA surface takes place when the organic phase is removed from the interface. Washing of the microspheres do not result in complete removal of PVA, leaving behind at least upto 8-12% of the stabilizer in the microspheres (Sahoo SK et al, 2002). This property of PVA causes a limitation in the amounts used for stabilizing the microsphere formulations.

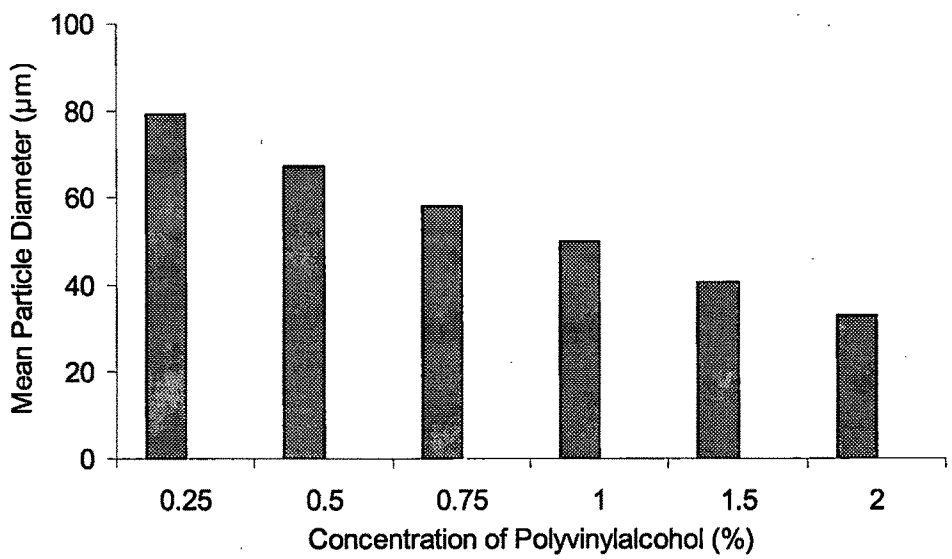


Figure 6.1: Mean particle size diameter of the ZB loaded PLGA 50:50 MS stabilized with various concentrations of poly vinyl alcohol (stirring speed – 1000rpm)

The concentration of PVA used for stabilizing the PLGA formulations may have a significant influence on their cellular uptake. It was found that 0.5% to 2% PVA did not show any effect, while at 5% concentration, a significant decrease in cellular uptake of PLGA nanoparticle formulations was reported (Sahoo SK et al., 2002). This is probably due to the increase in hydrophilicity of the formulation at high PVA concentrations. In the present study, 1% PVA concentration (within the range of concentration of PVA reported to not affect cellular uptake) gave a particle size of 48.15 ± 2.09µm for PLGA 50:50 MS. Further, these sizes of the microspheres were considered optimum as the size range of 25-180µm is suitable for intramuscular depot administration and preferably around 50µm (Mesens J et al., 2004). The

particle size of the PLGA 50:50 MS stabilized with different PVA concentrations are shown in figure 6.1.

3.3 Influence of stirring speed

The effect of different stirring speeds during the emulsification process (500 rpm to 2000 rpm) on the particle size of PLGA 50:50 MS were studied (Figure 6.2). Increase in the stirring speed lead to decrease in size of the microspheres. Higher stirring speeds result in high shear and kinetic energy thus prevents the particle agglomeration. Stirring speed of 750 rpm gave an optimum particle size of 48.15 ± 2.09μm for ZB loaded PLGA 50:50 MS suitable for intramuscular administration.

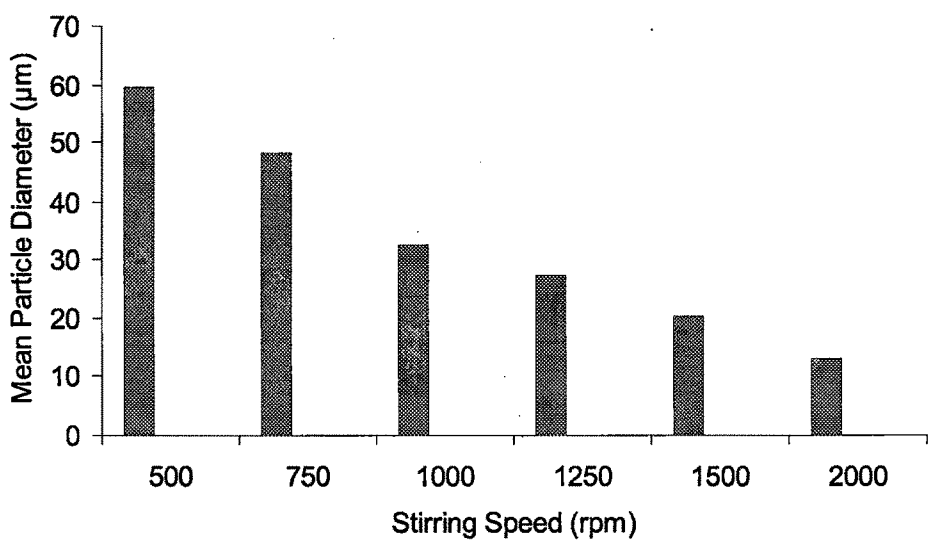
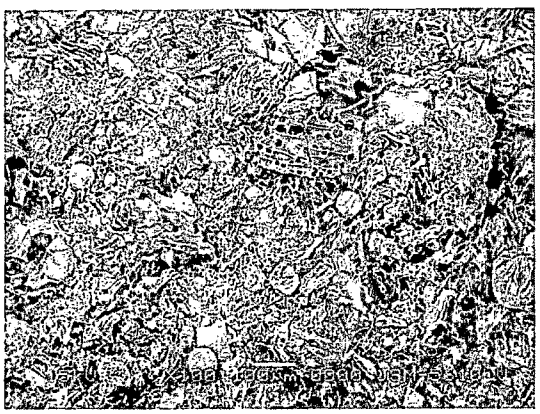


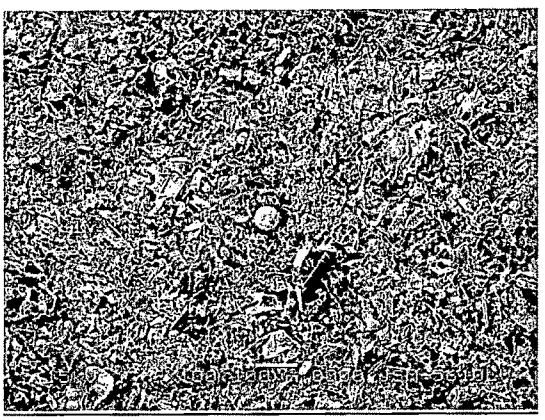
Figure 6.2: Mean particle size diameter of the ZB loaded PLGA 50:50 MS prepared at different shear rates (Poly vinyl alcohol concentration – 1.0%w/v)

Scanning electron microscopy

The scanning electron microscopy (SEM) revealed that the ziprasidone loaded PLGA 50:50 microspheres were spherical with a smooth surface (Figure 6.3A, B, C, D, E, and F). The SEM of the microspheres after dissolution for 35 days revealed that the microspheres were fragmented, irregular in shape and their surface was eroded (Figure 6.3G, H, I, J). Hence the drug release mechanism can be by surface erosion.

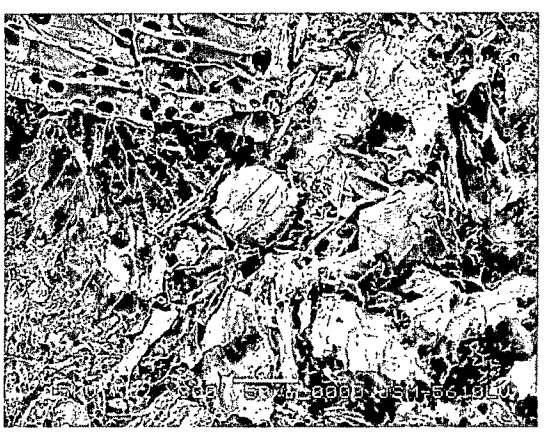


A



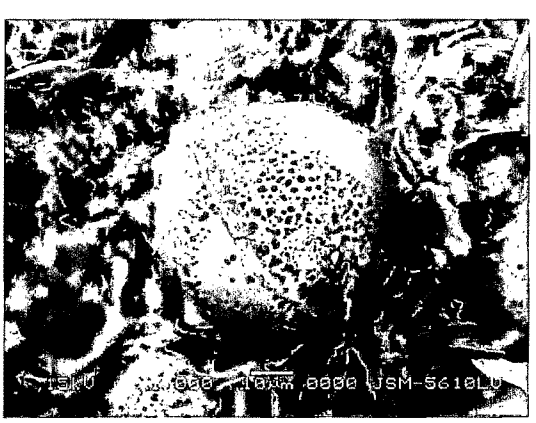
B

C



D

E



F

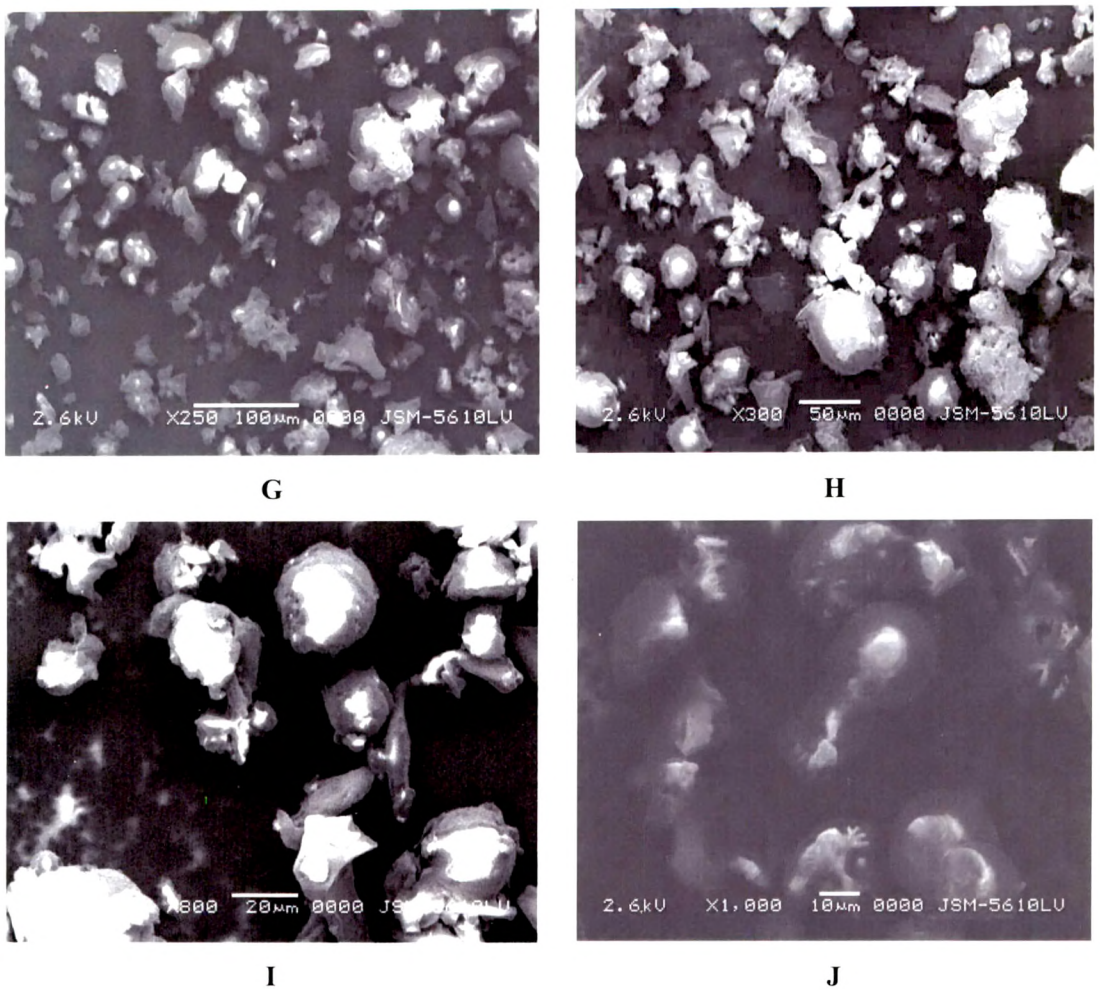
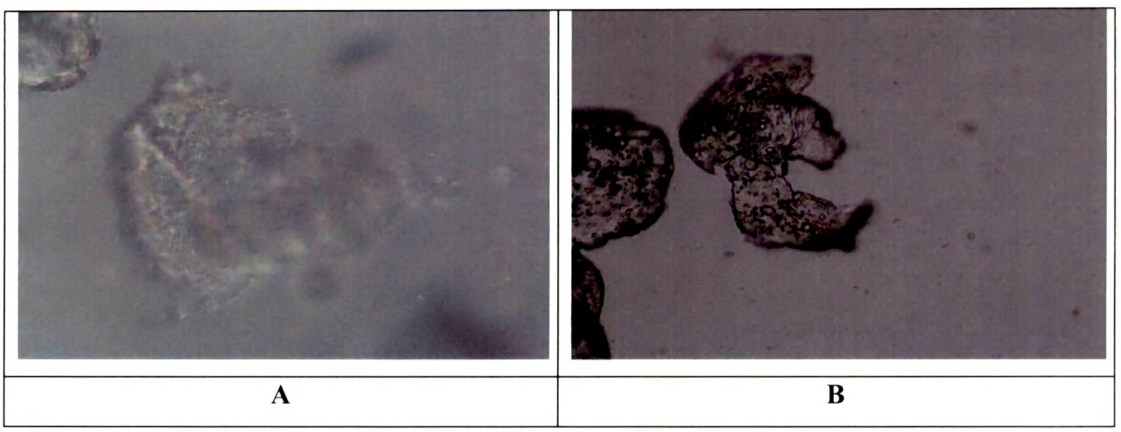


FIGURE 6.3: Scanning electron microscopy of Initial Ziprasidone loaded PLGA 50:50 at 100 X (A, B) and 1000 X (C, D, E, F). Scanning electron microscopy after 35 dissolution at 250 X (G), 300 X (H), 800X (I) and 1000 X (J).

Photomicrographs of the PLGA 50:50 MS after 35 days dissolution in phosphate buffer pH 7.4 containing 0.1% tween 80 also revealed signs of surface erosion (Figure 6.4A and 6.4B).



A B

Figure 6.4: Photomicrographs of ZB loaded PLGA 50:50 MS after 35 days dissolution

3.1 Effect of drug to polymer ratio

Six different batches of microspheres were prepared by varying theoretical drug loading % of ZB (5, 10, 15, 20, 25, and 30%). Increase in the polymer concentration lead to an increase in entrapment efficiency in the microsphere formulation. This can be explained by the fact that increase in the polymer content there is an increase in the accommodation of drug in the polymer matrix. Efforts to load drug in excess of 25% TDL led to decrease in the entrapment efficiency. This can be explained by the fact that 25% TDL led to saturation of the polymer matrix with ZB. The polymer matrix is no longer able to load or accommodate more ZB, hence leading to drop in the entrapment efficiency. The entrapment efficiency of ZB in the PLGA 50:50 MS are shown in Table 6.1.

S. No	Theoretical drug loading (%)	Entrapment Efficiency (%)	Particle Size (μm)
01	5	80.16	40.261
02	10	81.96	44.373
03	15	84.69	42.216
04	20	86.87	45.023
05	25	88.36	38.973
06	30	74.23	39.939

TABLE 6.1: Influence of theoretical drug loading on the particle size and entrapment efficiency of Ziprasidone

Differential Scanning Calorimetry

DSC analysis was performed for plain ziprasidone base (Figure 6.5a), PLGA 50:50 polymer (Figure 6.6b) and the ziprasidone loaded PLGA 50:50 microspheres (Figure 6.5c). The DSC curve of PLGA 50:50 polymer showed a glass transition temperature (*Tg*) of 56.64 °C. The DSC curve of plain ziprasidone base showed a melting endotherm for the drug at 227.99°C. This melting peak was absent in the DSC curve of the ziprasidone loaded PLGA 50:50 microspheres. This indicates the presence of ziprasidone in the amorphous form after entrapment into the PLGA 50:50 MS. The DSC curve also showed endotherms at around 53.99°C (Tg of PLGA 50:50) and 99.46°C (loss of residual water in the formulation)

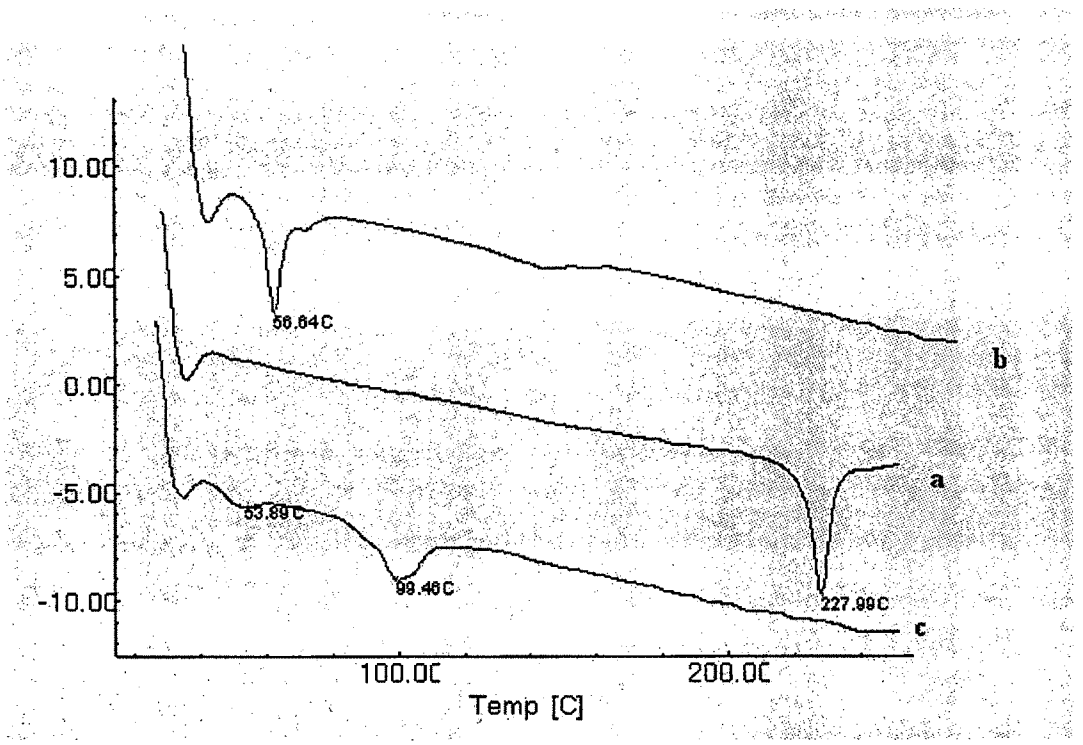


FIGURE 6.5: Differential Scanning Calorimetry thermograms of Ziprasidone base (a), PLGA 50:50 (b) and Ziprasidone loaded PLGA 50:50 (c).

X- ray diffraction studies (XRD)

X-ray diffraction studies of the plain ziprasidone base (Figure 6.6) revealed the crystalline nature of the drug, due to the presence of characteristic peaks was observed to reveal its crystallinity. X-ray diffraction studies of the ziprasidone loaded PLGA 50:50 microspheres (Figure 6.6) revealed the amorphous nature of the drug in the formulation, due to the absence of the characteristic peaks that was observed in the XRD of the pure drug.

The XRD pattern of ZB (Figure 6.6) shows a principal peak at angle 24.855° 2θ. In the ZB loaded PLGA 50:50 MS, the principal peak of ZB was absent (Figure 6.6). There was a reduction in the intensity of all the peaks in the microsphere formulation. This result indicates that ZB is in the amorphous state inside the PLGA 50:50 MS.

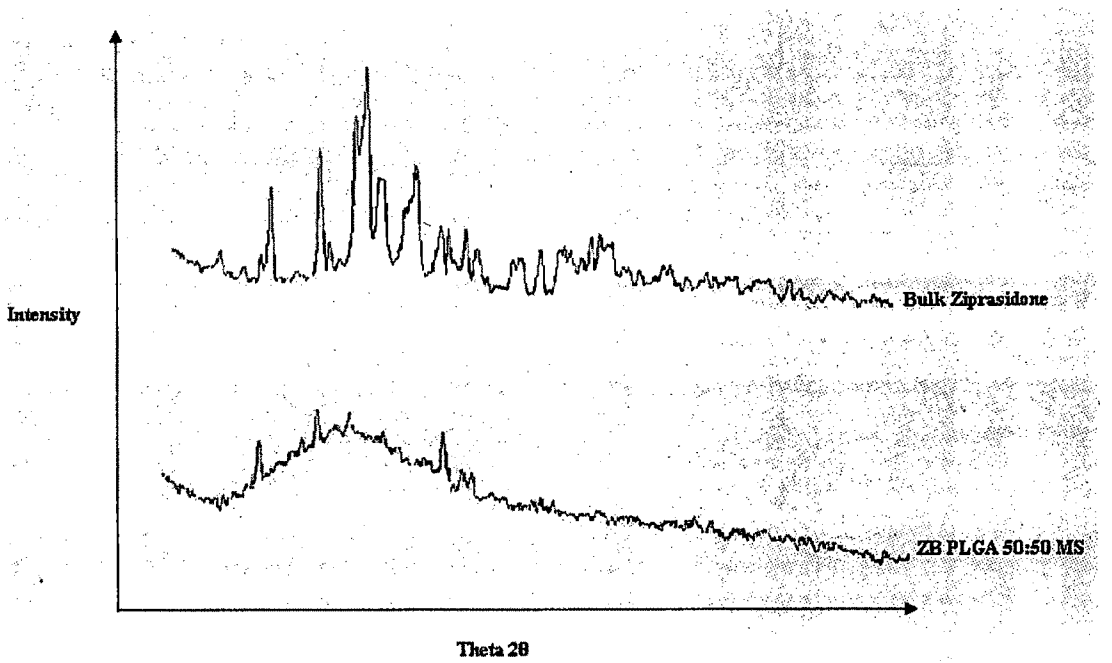


FIGURE 6.6: X – ray diffraction pattern of Ziprasidone base (a) and Ziprasidone loaded PLGA 50:50 (b).

In vitro release studies

The effect of different TDL on the initial drug loading was studied. There was a gradual increase in the initial burst release of ziprasidone (amount of ziprasidone released after day 1). PLGA microspheres with the maximum TDL (30%) gave the maximum initial burst release (17.96%) and PLGA microspheres with the minimum TDL (5%) gave the least initial burst release (8.13%). These results can be explained by the fact that increasing TDL is associated with saturation of polymer matrix with drug and there is an increase in the amount of surface adsorbed drug. The percentage drug released versus the square root of time was plotted after omitting the initial time points corresponding to the burst release. Straight lines were obtained indicated that the release was diffusional in nature according to the Higuchi model. The slopes of the straight line obtained are proportional to an apparent diffusion coefficient (Siepmann J and Peppas NA., 2001). There was a gradual increase in the diffusion coefficient values with increase in the TDL. The values are tabulated in table 6.2.

The general mechanism of drug release from biodegradable polymer matrixes can be probably due to the release of drug from the pores created upon erosion of the polymer matrix. For PLGA matrices, diffusion of drug through pre-existing pores and channels in the microsphere matrix that subsequently become interconnected and enlarged, as chemical degradation of the polymer back bone takes place, has been regarded as a predominant drug-release mechanism (Shah SS et al., 1992). The above factors lead to the increased porosity of the microparticles and mass erosion. Another probable mechanism could be the hydrolysis of polymer occurring with time and hence the erosion of microspheres leading to increased drug release from the polymer matrix (Jeyanthi R et al., 1997). Drug release from the PLGA matrix systems is believed to be by hydrolytic degradation of the polymer matrix and also by diffusion of drug through the polymer matrix. This is further influenced by a number of factors namely the molecular weight, crystallinity and the ratio of lactide to glycolide of the PLGA copolymer.

In order to study the drug release kinetics from the polymeric microspheres, the data obtained from the in vitro drug release experiments were fitted into the following equation (Siepmann J and Peppas NA et al., 2001)

$$M_t/M_\infty = M_b/M_\infty + kt^n$$

where,

M_t = amount of drug released in time "t"

M_∞ = amount of drug released in infinite time

M_b = amount of burst released drug

k = release constant

n = release exponent

The above equation fitted the experimental data well. It was assumed that there was no burst release of drug i.e. the phase I due to the initial burst release of drug was not considered. In that case M_b/M_∞ becomes zero at zero time. The value of n depends on the geometry of the release device and the mechanism of release. For spheres, n ranges between 0.43 if the drug release is diffusion-based (Fickian transport) and 0.85 if the drug release is degradation-based (Case II transport). If the n values fall between 0.43-0.85, the release mechanism is said to be of anomalous non Fickian type (Siepmann J and Peppas NA et al., 2001)

In case of Fickian transport the release of the drug from the polymer matrix system is a function of rate of water penetration inside the polymer matrix from the vicinity and the rate of drug diffusion. In Case II transport the diffusion coefficient depends on rate of degradation of the polymer chains. Some systems show a diffusion pattern, which is a mixture of Fickian and Case II diffusion and is usually termed as "anomalous diffusion". Polymer degradation is one of the mechanisms of drug release from biodegradable polymer matrixes. Several reports are available wherein the drug release from PLGA microspheres has been found to be dependent on the rate of surface erosion. (Lin SY et al., 2000). Lin and co workers showed that release of diclofenac sodium from PLGA and PLA microspheres followed first order release kinetics. As per Wagner's theory, first order release kinetics is seen in devices whose surface area changes exponentially with time.

In the present investigation, it can be inferred from the in vitro release pattern that drug release due to polymer degradation may be prominent only during the later stages of the dissolution study (after 35 days) as we have not observed any second burst release due to polymer degradation during the present period of study. The plot of percentage drug release versus square root of time gave a liner fit for all the three polymer microspheres indicating that the drug release is primarily due to Higuchi's diffusion kinetics. The scanning electron micrographs (Figure 6.3G, H, I and J) and photomicrographs (Figure 6.4A and 6.4B) of the microspheres taken at the end of 35 day dissolution study revealed low signs of microsphere surface degradation. Further, there was no significant change in the particle size visually before and after in vitro dissolution testing for 35 days. The rate of hydrolysis of the polymer chain depends on the changes in pH, presence of catalyst and temperature. PLGA 50:50 has the fastest in vivo degradation rate (50-60 days) of the various D, L lactide – glycolide materials (Bala I et al., 2004). In the present study, polymer degradation in the dissolution media used (phosphate buffered saline pH 7.4 containing 0.1% tween 80) is not apparent until the time period (35 days) studied. The invitro release of the PLGA 50:50 MS with different TDL are shown in figure 6.7.

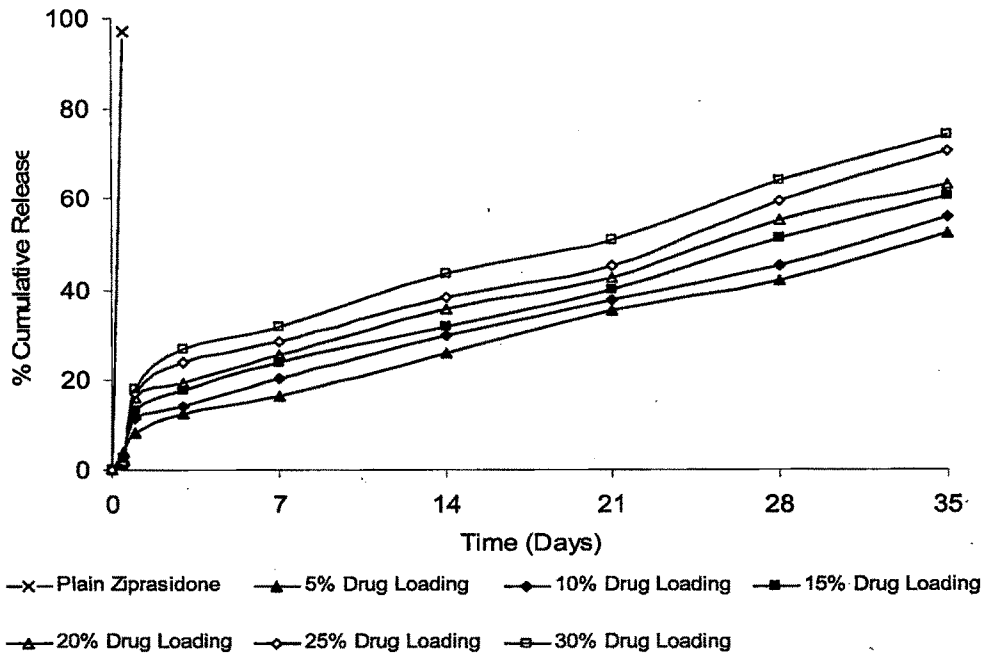


FIGURE 6.7: In vitro release of ZB from the various TDL loaded PLGA 50:50 microspheres.

Theoretical drug loading (%)	Higuichi's Regression coefficient (R^2)	Apparent Diffusion Coefficient (Slope)
5	0.9828	9.455 ± 0.35
10	0.9808	9.619 ± 0.56
15	0.9778	9.962 ± 0.51
20	0.9824	10.407 ± 0.37
25	0.9794	10.934 ± 0.24
30	0.9763	11.286 ± 0.49

TABLE 6.2: Kinetic evaluation of the in vitro release of ZB from the PLGA 50:50 microspheres loaded with different amounts of ZB in phosphate buffer pH 7.4 containing 0.1% Tween 80.

Stability studies

There was no significant change in the particle size of the lyophilized ZB loaded PLGA 50:50 MS in both the storage temperatures studied for six months. There was no significant change from the initial release pattern during the time period studied. The results of the stability evaluation are given in table 6.3. There were no signs of particle agglomeration when the microspheres were examined by powder microscopy (OLYMPUS DP-12). The time required for reconstitution was almost similar at both the temperatures throughout the time period studied (20 to 33 seconds).

In vitro release studies were carried out for the microspheres removed after 1, 2, 3 and 6 months 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 f_1 value was 8.8 at the end of 6 months for the microspheres stored at 4°C and 14.1 for the microspheres stored at room temperature. The f_2 was 89.7 at the end of 6 months for the microspheres stored at 4°C and 78.2 for the microspheres stored at room temperature. These values denote that there is not much significant change in the release pattern of ZB from the PLGA 50:50 microspheres on storage.

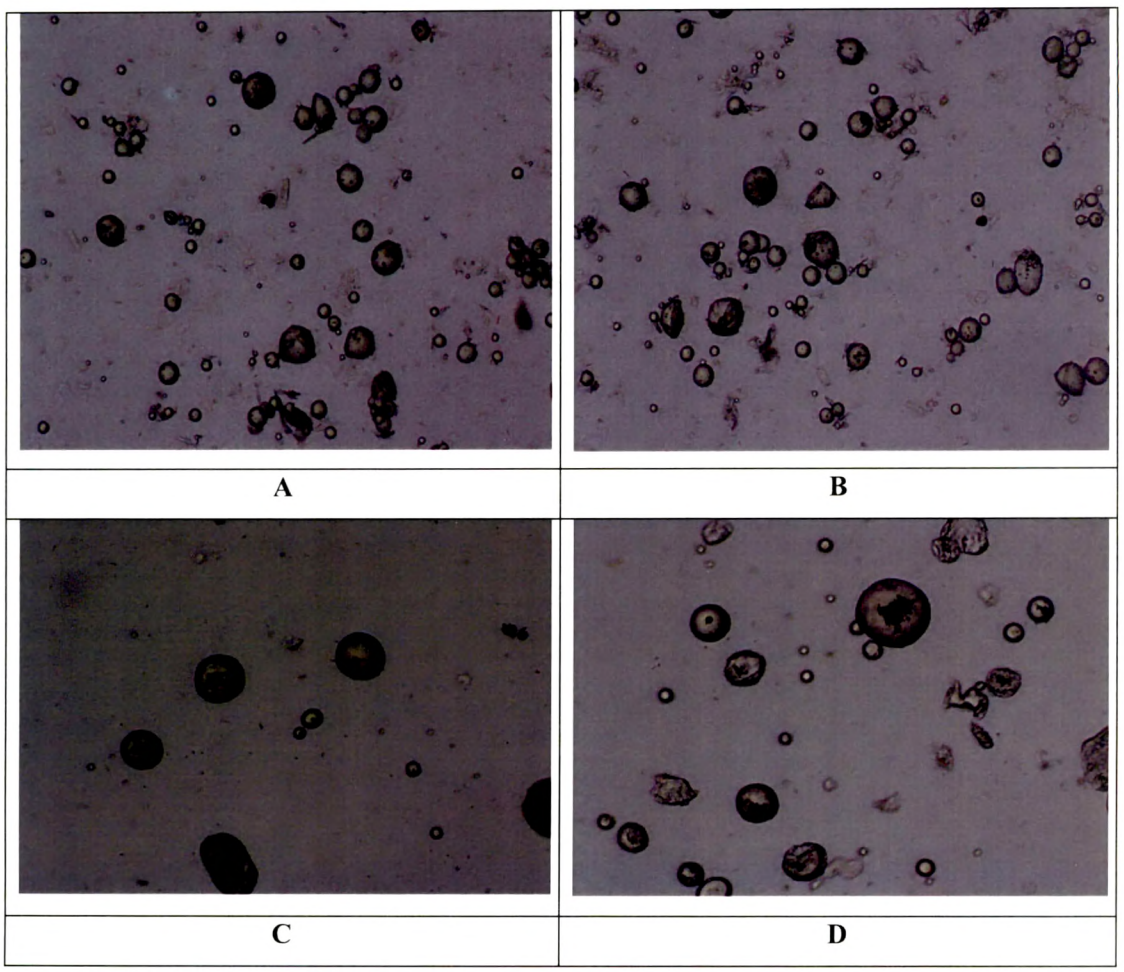


FIGURE 6.8: Photo micrographs of ZB loaded PLGA 50: 50 MS after 3 months storage at 4°C (A, C), Photo micrographs of ZB PLGA 50: 50 MS after 6 months storage at ambient room emperature {27 – 30°C} (B, D)

Time (months)	Samples stored at 4°C				Samples stored at 30°C			
	Particle Size (μm ± SEM)	Time for reconstitution (seconds ± SEM)	In vitro release		Particle Size (μm ± SEM)	Time for reconstitution (seconds ± SEM)	In vitro release	
			F1	F2			F1	F2
1	39.25 ± 2.31	20 ± 2.06	4.1	94.5	38.64 ± 2.02	30 ± 1.25	5.7	90.4
2	41.78 ± 1.65	22 ± 1.40	4.6	90.1	42.32 ± 1.59	29 ± 1.84	8.2	86.5
3	40.74 ± 1.31	23 ± 1.58	5.9	88.3	40.46 ± 1.40	31 ± 1.09	9.1	81.6
6	42.24 ± 1.39	25 ± 1.86	7.8	82.7	39.96 ± 1.23	33 ± 1.61	10.0	74.2

TABLE 6.3: Mean particle diameter, time for reconstitution and the in vitro release pattern of ZB loaded PLGA 50:50 MS stored at different temperature conditions in dark condition. The values are average of three determinations.

6.4 Conclusions

Ziprasidone (ZB) loaded poly lactide co-glycolide 50:50 (PLGA 50:50) microparticles were prepared by emulsification and solvent evaporation technique. The maximum theoretical drug loading (TDL) possible was 25%. DSC and XRD revealed change in crystallinity of ZB after incorporation into PLGA 50:50 MS. SEM photographs revealed that the MS were spherical in shape and smooth in texture. The ZB loaded PLGA 50:50 MS showed sustained release in vitro upto 35 days. There was no significant change from the initial values in the particle size, reconstitution time and in vitro release. These data substantiate the potential of the above formulated PVA stabilized ZB loaded PLGA 50:50 MS as a potential long acting antipsychotic formulation.

6.5 References

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