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# Encapsulation of olanzapine into Waxes/fat microspheres: Preparation, Characterization and Release kinetics

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**ABSTRACT**
The objective of the present study was to minimise the unwanted side effects of olanzapine (OZ) drug by kinetic control of drug release by entrapping OZ into gastro resistant, biodegradable waxes such as beeswax (BW), cetostearyl alcohol (CSA), spermaceti ( SP) and fat cetyl alcohol ( CA) microspheres using meltable emulsified dispersion cooling induced solidification technique utilizing a wetting agent. Solid, discrete, reproducible free flowing microspheres were obtained. The yield of the microspheres was up to 94.0%. Microspheres had smooth surfaces, with free flowing and good packing properties, indicating that the obtained angle of repose, % Carr's index and tapped density values were well within the limit. More than 97.0% of the isolated spherical microspheres were in the particle size range of 312-330 µm as confirmed by scanning electron microscopy (SEM) photographs. The drug loaded in microspheres was found to be stable and compatible with waxes as confirmed by DSC and FTIR studies. The release of drug was controlled for more than 8 h. Intestinal drug release from microspheres was studied and compared with the release behaviour of commercially available formulation Olanex®. The release kinetics followed different transport mechanisms. The drug release performance was greatly affected by the materials used in microsphere preparations, which allows absorption in the intestinal tract.



## Introduction

Olanzapine (OZ) is a novel antipsychotic agent, has a pleotrophic pharmacology and affects dopaminergic, serotonergic, muscarinic and adrenergic activities[1]. Clinical studies and trials suggest OZ is superior to haloperidol and also maybe superior to risperidone in terms of efficacy and side-effect profiles[2].The therapeutic advantage of recent antipsychotics (so-called atypical antipsychotics) has been attributed to alpha-2 adrenergic antagonist effects[3].The starting dose of OZ is a single evening dose of 10mg. The usual maximum dose should be 20mg. It is practically insoluble in water, having only 60% oral bioavailability[4]. OZ undergoes extensive first pass metabolism. The dosage of OZ varies depending upon the reason for its use. When used to treat schizophrenia, 5–10 mg is the typical starting dosage. If dosage adjustments are needed, increases are made in 5-mg increments once a week. During treating schizophrenia, a total daily dosage of 10–15 mg is usually effective and 20 mg per day may be needed for maximum effect. The safety of doses greater than 20 mg per day has not been determined[5]. OZ is eliminated from the body more quickly in young people than in older (over age 60) individuals, in men than in women, and in smokers faster than in non-smokers[6]. Dosage adjustments may be needed based upon individual patient characteristics. Considering the long regimen of anti maniac therapy, the administration of OZ was reported to induce adverse side[7- 9]. Side effects that occur in more than 5% of patients taking OZ include involuntary movements, weakness, dizziness, extreme drowsiness, nonviolent objectionable behavior, constipation, weight gain, dry mouth, low blood pressure, stomach upset, increased appetite, cold-such as symptoms, or fever.Other side effects that are possible include rash, body aches and pains, elevated liver enzymes, vision abnormalities, chest pain, or rapid heartbeats, tardive dyskinesia, neuroleptic malignant syndrome. To achieve maximum therapeutic effect with a low risk of adverse effects, controlled released preparations are preferred [10]. The side effects could be lowered by controlling the drug release and by adjusting the absorption rate. This can be achieved by employing suitable modifications in the manufacturing process[11]. Delivering the drug in the intestinal milieu from wax microspheres could be manipulated by suitable coating techniques [12]. The chief characteristics of enteric coating are their impermeability to gastric juices but susceptibility to intestinal juices[13,14]. OZ should be dosed at least two doses with a maintenance dose per day. Due to its low therapeutic index, the frequency of adverse effects may be dose related [8]. A controlled release dosage form is preferable than the conventional dosage form, because there is a considerable saving in nurses and pharmacists time. As demonstrated by pharmacokinetic studies on OZ, the ingestion of a single controlled release formulation is effective even when administered once a day [3,5]. These findings suggested that kinetic control is effective for preventing the of toxicity of OZ. Previous experimental results demonstrated that waxes are biocompatible, non-immunogenic material used for the entrapment of drug, used for controlling drug release in the intestinal tract [13.14]. The objectives of the present study are to formulate, characterize and study the *in vitro* drug release from wax/fat microspheres loaded with OZ. The pattern of drug

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release from the wax/fat microspheres is compared with that of the commercially available enteric coated oral formulation Olanex® - 10 mg tablet.

**Materials and Methods**

Olanzapine was obtained as a gift sample from Ranbaxy, Mumbai, India. Beeswax, cetostearyl alcohol, spermaceti, cetyl alcohol, span 20 and Tween 80, all the other chemicals and solvents used were of analytical grade, purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

**Preparation of wax microspheres**

9 gm of waxes (BW, CSA, SP) and fat (CA) were melted separately in china dish using water baths. Drug (3 gm) previously passed through sieve no.100 was dispersed in the melted wax/fat mass and stirred to obtain a homogeneous melt. These individual mixtures were poured into 200 ml of pH 7.2 phosphate buffer solution (to minimize the solubility of drug), which was previously heated to a temperature higher than melting point of wax (>+ 5°). Tween 80 (1.7 % w/w) was added to the mixture containing waxes and span 20 (1.9% w/w) for the mixture containing fat. The whole mixture was mechanically stirred at 800 rpm using a stirrer (RQ-127A) fitted with a 4-blade impeller of approximately 53 mm diameter. Spherical particles are produced due to dispersion of molten wax in the aqueous medium. The mixture was stirred continuously at 800 rpm at a higher temperature (>+ 5°) of the melting point of waxes/fat for 4 min. The temperature of the mixture in the beakers was cooled rapidly to 10° C by the addition of cold water. The resultant solid spheres collected by filtration were extensively washed with water to remove any drug and surfactant residues. Air drying was carried out at room temperature for 48 h produced discrete, free flowing solid microspheres.

**Size analysis of microspheres**

The separations of the microspheres in to various size fractions were carried out by sieve analysis technique and SEM analyzed the size of microspheres.

**Micromeritic properties**

Tap density of the prepared microspheres was determined using tap density tester and % Carr's index (% I ) was calculated. Angle of repose was assessed to know the flowability of wax microspheres.

**Scanning electron microscopic studies and sphericity determination**

SEM photographs were taken using scanning electron microscope Model Joel- LV-5600, USA, at suitable magnification at room temperature. The photographs were observed for morphological characteristics and to confirm spherical nature of the microspheres. To determine the sphericity, the tracings of wax/fat microspheres (magnification 45 X) were taken on a black paper using Camera Lucida, (Model-Prism type, Rolex, India) and circulatory factor was calculated [19]. The sphericity of microspheres was calculated using the equation,

$S = p^2/(12.56 \times A)$. where A is area ($cm^2$) and p is perimeter (cm) 1

**Differential scanning calorimetry (DSC)**

All dynamic DSC studies were carried out on DuPont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with the help of an empty cell (high purity alpha alumina discs of DuPont Company) as the reference. The instrument was calibrated using high purity indium metal as standard. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10°/min. The runs were made in triplicate.

**Fourier transform infrared spectroscopy (FTIR)**

FTIR spectra of pure drug, empty microspheres and drug loaded microspheres were obtained using KBr pellet method (applying 6000 kg/$cm^2$). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, Model 8033,USA)in the wave number region 400 - 4000$CM^{-1}$ to drug excipient interactions.

**X-ray diffractometery (XRD)**

X-ray diffraction patterns of pure OZ and drug loaded waxes/fat microsphere were recorded using (Phillips PW 1710, Tokyo, Japan) X-ray diffractometer with a copper target, voltage 40 Kv, current 30 MA at a scanning speed of 0.30°C /min.

**Loose surface crystal study (LSC)**

This study was conducted to estimate the amount of drug present on the surface of the pellets. 100 mg of pellets was suspended in 100 ml of phosphate buffer (pH 7.4). The samples were shaken vigorously for 15 min in a mechanical shaker. The amount of drug leached out from the surface was analyzed spectrophotometrically at 274 nm. Percentage of drug released with respect to entrapped drug in the sample was recorded.

**Estimation of drug loading**

Drug incorporated wax microspheres of each batch was selected and powdered in a mortar. 100 mg of drug loaded waxes/fat microspheres was accurately weighed and added in to 100 mL volumetric flask. To this, 100 mL ethanol was added. This solution was stirred for 60 min, till the entire drug leached out. The solution was filtered and 1 mL was withdrawn from this solution and added in to 10 mL volumetric flask and volume was made to 10 mL(10 µg/mL) with phosphate buffer pH 6.8. Drug content was estimated UV spectrophotometrically at 274 nm.

***In vitro* studies**

USP XX1 dissolution apparatus type II was employed to study percentage of drug release from various formulations prepared. Accurately weighed quantities of drug (OZ 10 mg equivalent to a commercial preparation – Olanex®10 mg tablet,) loaded microspheres of each batch were taken in 900 ml dissolution medium ( 2 h in pH 1.2 hydrochloric acid buffer and 6 h in pH 7.4 phosphate buffer) and stirred at 100 rpm by maintaining at a temperature of 37 °C ± 0.5°. The drug concentrations were determined by withdrawing the 10 ml of aliquots using guarded sample collectors periodically at an interval of 30 min for first 4 h and at 60 min interval for the next 4 h. Release studies were carried out in triplicate. A differential factor ($f_1$) and similarity factor ($f_2$) were calculated from dissolution data according to the following equations;

$$f_1 = \frac{\sum_{t=1}^{n}(R_t - T_t)^2}{\sum_{t=1}^{n} R_t} \times 100 \quad (1)$$

$$f_2 = 50 \log \left\{ \left[ 1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

where, $f_1$ - differential factor, $f_2$ - similarity factor, n – number of time point, $R_t$ – dissolution value of the reference at time, 't' and $T_t$ - dissolution value of test formulation at time 't'. Differential factor, $f_1$ was calculated by the percentage difference between the two curves at each time point and

measured the relative error between the two curves. The acceptable range for differential factor, $f_1$ is 0-15. The similarity factor, $f_2$ was logarithmic reciprocal square root transformation of the sum-squared error and is a measure of the similarity in the percentage dissolution between the reference and test products. If dissolution profile to be considered similar, the values for $f_2$ should be in the range 50 - 100.

**Stability studies**

The optimized formulation was subjected to stability studies. The stability studies were carried out by storing the microspheres in capsules kept in a glass bottle at 25 $^0$C / 60 % RH30 $^0$C / 65 % RH and 40 $^0$C / 75for 90 days. These samples were collected on 15$^{th}$ , 45$^{th}$ and 90$^{th}$ day, checked at regular intervals for changes in physical appearance. Drug content was estimated UV spectrophotometrically at 274 nm.

**Results and Discussions**

Evidence have shown in the recent years that waxy/fat materials have the physical properties and behavior suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen[15].

In the present study, a modified novel meltable dispersion emulsified cooling induced solidification method was employed using inert waxes/fat (FDA Approved) and non-toxic solvents to entrap the drug. In the present study, various parameters were studied such as drug and wax ratio, stirring speed and time, amount of surfactant added, volume of the aqueous phase used, effect of pH on drug entrapment, temperature of the aqueous phase and rapid cooling studies. Therefore the influence of the above parameters was highlighted. When the pH value of the external aqueous phase was highly alkaline, the solubility of the drug was reduced and the encapsulated amount of the drug increased. The maximum drug load wasobtained at pH 7.4. When pH value changes from 7.4 to 5.0, the percent of drug loading reduced from 12.28 to 2.9 %, 13.89 to 2.8 % , 12.78 to 3.0 % , 12.12 to 2.7 % for $F_1$( BW), $F_2$ ( CSA) , $F_3$ (SP) and $F_4$ (CA) formulations

In the present study, it was found that 200 ml of aqueous phase suitable for producing the spherical microspheres. Resultant microspheres did not have any surface irregularities and are non aggregated. As the volume of external phase increased, the yield was reduced and the resultant microspheres were irregularly shaped. When the volume of the aqueous phase was less than 150 ml, the resultant microspheres were highly aggregated in nature and highly impossible to distinguish as individual microspheres. In order to avoid the formation of irregularly shaped larger particles, in the present method, 200 ml of aqueous phase was used.

Incorporation of OZ into BW, CSA, SP and CA microspheres required the addition of tween 80 as a surfactant and span 20 for SP , at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. An attempt was made to incorporate drug in the wax microspheres without the addition of a surfactant. But the process failed, as it resulted in an aggregate cake like mass during the solidification of wax. This may be due to repulsion resulting from high interfacial tension between the hydrophobic waxy material and external aqueous phase. It was found that tween 80 having a HLB value of 15 was suitable to increase substantially dispersion of waxy material in external aqueous phase and promote drug incorporation in the wax microspheres. To obtain an optimal surfactant concentration, various concentrations ranging from 1.2 to 2.2 % (w/w) of the total formulation were tested. Discrete microspheres with good flow properties using an optimum concentration of surfactant 1.7 % w/w (tween 80) for BW, CSA, SP and CA and 1.9 % w/w span 20 for SP were used. Concentrations of tween 80 ranging from 1.2 to 1.6 % w/w and 1.2 to 1.8 % w/w/of span 20 failed to produce reproducible microspheres.The resultant waxes/fat microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres. A similar surfactant concentration was reported for wax microspheres prepared by meltable dispersion method[16].

Temperature of the aqueous phase was maintained at 5 $^0$ C higher than the melting point of the waxes/fat in the corresponding formulations. From SEM studies it was observed that the resultant microspheres were free from surface irregularities, except some wrinkles. It was also observed that when the temperature of the aqueous phase was less than the 5 $^0$ C than the melting point, a big waxes/fat flakes were produced.

In the present study, to produce the spherical discrete microspheres, an optimum drug to waxes/fat phase ratio of 1:3 w/w was used. It was found that higher the amount of drug to wax ratio (2:3) produces aggregate masses during the cooling process. It may be due to reduced melting point of the waxy/fat materials. SEM photographs also indicated the presence of the crystals on the surface of the microspheres. The resultant microspheres were unsuitable for pharmaceutical uses. Hence an optimum 1:3 ratio was used to prepare microspheres.

Sieve analysis data obtained for prepared waxes/fat microspheres were in the size range of 106 to 500 µm and 58.43 to 64.12 % were of size fraction 250 µm. It was observed that the average size of the microspheres ranged between 312 to 320 µm. The important factor that influences the size distribution of microspheres is the optimum stirring speed and stirring time.

A stirring speed of 800 rpm and stirring time of 4 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 800 to 1200 rpm there was a decrease in the average size of the spheres and recovery yield of the microspheres, due to small sized microspheres, which were lost during successive washings. When the stirring speed was lower than 800 rpm, larger pellets were a formed. It was also found that an increase in stirring time, from 5 to 8 min (at a stirring speed of 800 rpm), there was a decrease in the recovery yield of microspheres. When the stirring time lower than 4 min, melted waxes/fat materials adhered to the sides of the beaker during the cooling process, resulted in lower recovery of yield.

Micro particulate drug delivery systems are formulated as single unit dosage forms in the form of capsule or tablet. Such microparticulate systems should possess the better and adequate micromeritic properties [17]. The obtained micromeritic properties are given in Table 1.The values of angle of repose were well within the range, indicating reasonable good flow potential for the microspheres. The tapped density values ranged between 0. 43 g/cm$^3$ to 0. 48 g/cm$^3$. The results of % compressibility index ranges from 10.45 % to 12.34 %, suggests good flow characteristics of the microspheres [Table 1]. The better flow property indicates reasonable and good flow potential of prepared microspheres.

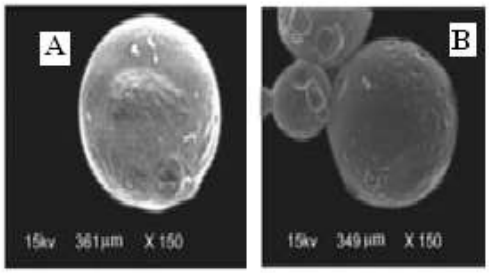


**Figure1. SEM photomicrographs of; (A) OZ loaded microsphere in spherical shape (F2), (B) OZ loaded microspheres showing inward dents and shrinkage (F2)**

SEM photographs showed that the wax/fat microspheres were spherical in nature, had a smooth surface with inward dents and shrinkage, which is due to the collapse of the wall of the microspheres Fig.1. photograph reveal the absence of crystals of the drug on the surface of microsphere, indicating uniform distribution of the drug within the microspheres and further indicate that low molecular weight waxes produce better quality microsphere than that of high molecular weight waxes. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product[18]. The sphericity factor obtained for the microspheres nearer to the value 1, confirming the sphericity of the microsphere.

DSC studies were performed on pure drug and drug-loaded waxes/fat microspheres have shown sharp endothermic peaks. OZ exhibits a sharp endothermic peak at 196.68° C presented in fig. 2. It was observed that presence of the endothermic peak of the drug at 195.32°C (F2) 194.23°C (F1) 194.15°C (F3) & 193.41°C (F4) in the drug loaded waxes/fat microspheres indicates, that the drug is uniformly distributed in the microspheres. The peak intensity corresponding to the melting of OZ decreased in the thermograms of OZ loaded waxes/fat microspheres. These results indicate that only a small fraction of the drug substance existed in the crystalline state. The presence of melting endotherm was in the OZ loaded waxes/fat microspheres, which indicates that OZ was completely dispersed inside the waxes/fat microspheres. Reduction in the melting point and enthalpy of the melting endotherm was observed in the OZ loaded waxes/fat microspheres. Small sized waxes/fat microspheres leads to high surface energy, which creates an energetically suboptimal state causing a decrease in the melting point[19].

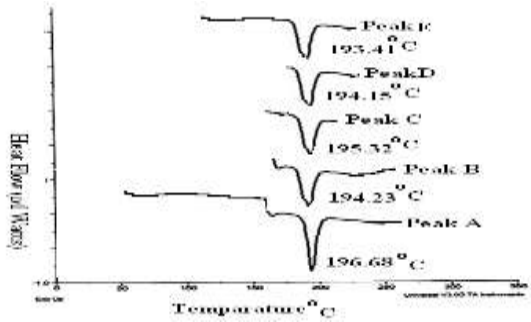


**Figure 2. DSC thermograms of pure OZ and OZ loaded waxes/fat microspheres, peak A= Pure OZ, peak B = F1, peak C = F2, PeakD = F3 & peak E=F4 *OZ = Olanzapine,F1= OZ& Beeswa, F2 = OZ & Cetostearyl alcohol, F3 = OZ & Spermaceti, F4 = OZ & Cetyl alcohol***

From the FTIR studies, the characteristic bands for important functional group of pure drug OZ, empty microspheres and drug-loaded waxes/fat microspheres were identified. It was observed that 2932.10 $cm^{-1}$ due to C-H stretching, 1586.10 $cm^{-1}$ due to C = C stretching, 1559.21 $cm^{-1}$ due to C = N stretching, 1225.78 $cm^{-1}$ due to N-H stretching, 1033.96 $cm^{-1}$ due to O-H bending and 745.88 $cm^{-1}$ due to C-S bending. FTIR spectra showed that the characteristics bands of OZ were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and waxes/fat used. A comparison and interpretation of this region in our spectra agrees with their conclusions[20].

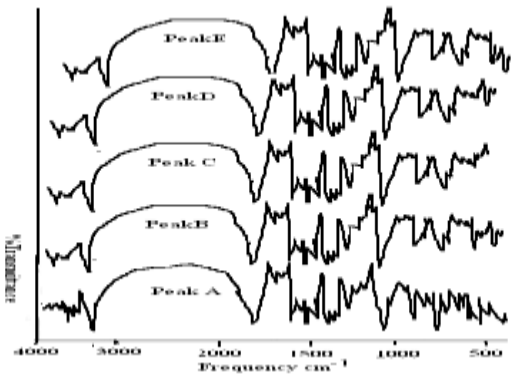


**Figure 3. FTIR spectra of pure OZ and OZ loaded waxes/fat microspheres, peak A= Pure OZ, peak B = F1, peak C = F2, Peak D = F3 & peak E=F4 *OZ = Olanzapine,F1= OZ& Beeswax, F2 = OZ & Cetostearyl alcohol, F3 = OZ & Spermaceti, F4 = OZ & Cetyl alcohol***

X-RD pattern of pure OZ showed principal peak at 20.63° and intense peaks at 8.45°, 18.19°, 19.71°, 21.42°, 23.78° , OZ loaded waxes/fat microspheres showed principal peak at 20.73°& intense peak at 8.39°, 18.62°, 21.44°, 22.98°, 23.65° to F1, principal peak at 20.76° & intense peaks at 8.53°, 19.72°, 21.22°, 22.19°, 23.56°, 20.65° to F2, principal peak at 8.59°, & intense peaks at 19.74°, 21.51°, 22.11°, 23.93° 9 20.95° to F3, principal peak at 8.50° & intense peaks at 19.02°, 21.32°, 22.71°, 23.65°, 20.82° to F3, principal peak at 8.48°& intense peaks at 19.12°, 21.34, 22.33°, 23.60° to F4 respectively, as presented in Fig.4. X-ray diffraction patterns revealed the crystalline nature of pure OZ. X-ray diffractogram of OZ showed number of sharp and intense peaks. But Increased peak width was observed in XRD pattern of OZ loaded waxes/fat microspheres.X-ray diffractogram of OZ loaded waxes/fat microspheres (F1, F2, F3 & F4) showed broad peaks with low intensity. This may be attributed to the incorporation of OZ between parts of the crystal lattice of the beeswax, cetostearyl alcohol, spermaceti & cetyl alcohol , leading to a change in the degree of crystallinity of the OZ [21].

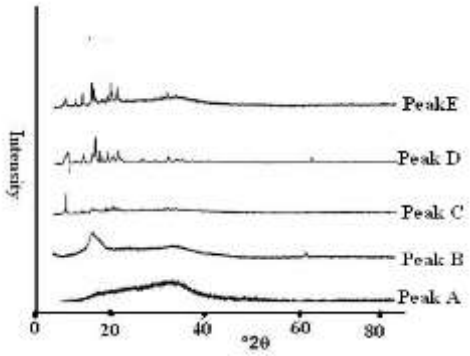


**Figure 4. X-ray powder diffraction patterns of pure OZ and OZ loaded waxes/fat microspheres, peak A= Pure OZ, peak B = F1, peak C = F2, Peak D = F3 & peak E=F4**

***OZ = Olanzapine, F1= OZ& Beeswax, F2 = OZ & Cetostearyl alcohol, F3 = OZ & Spermaceti, F4 = OZ & Cetyl alcohol***

Loose surface crystal (LSC) study is an important parameter giving indications of the amount of drug on the surface of the waxes/fat microspheres. Physical state of OZ in all formulations with different drug loading was investigated by polarized light microscopy. Microscopic studies indicated that crystalline OZ was observed clearly in formulation F2 ( Drug loading was 13.89 % w/w) than other formulations.

Drug loading and drug encapsulation efficiency of the drug loaded waxes/fat microspheres (F1, F2, F3 & F4) are given in the Table 2. Drug content in all the formulations were in the range of 12. 12 to 13.89 % w/w. Drug content was least in formulation F4 (12.12 % w/w) and high for formulation F2 (13.89 % w/w). Drug encapsulation efficiency was found to be more in formulation F2 (3.93 % w/w/) and less in formulation F4 (81.10 % w/w). Interestingly drug content and drug encapsulation efficiency increases with increasing in waxes/fat size (312 to 320 μm). This might be due to increased relative surface area of the pellets, leads to more drug content and drug encapsulation efficiency.

From the release studies it was observed that, no significant release of drug at gastric pH from waxes/fat microspheres. At the end of 8$^{th}$ h, *in vitro* drug release from F2 (94.32%), F1 (86.32 %), F3 (85.12 %) & F4 (87.12 %) was slower than Intalith CR®- 450 (97.12%) in the intestinal environment. Drug was released in a biphasic manner consisting of initial fast release followed by a slow release in intestinal pH from the waxes/fat microspheres [22]. The decreased *in vitro* drug release from waxes/fat microspheres might be due to more hydrophobicity and influence of molecular weight of waxes/fat. The *in vitro* drug release was considerably retarded from the wax microspheres when compared Olanex®– 10 mg tablet. The rate of drug release followed first order release kinetics and numerical data fitted into Peppa's model showed that, the mechanism of drug release from waxes/fat microspheres was non fickian diffusion. After an initial burst effect, the subsequent release of drug from microspheres was slow, and the influence of molecular weight (MW) was observed. The rate of drug release followed first order kinetics and numerical data fitted into Peppas' equation. Statistically estimated values of n of drug from pellets at 95 % confidence limit, is in the range 0.34 to 0.42 for waxes/fat microspheres (F1, F2,F3 &F4) studied and 0.41 for Olanex®– 10 mg tablet, indicated that the drug release from the waxes/fat microspheres(F1, F2,F3 &F4) and Olanex®– 10 mg tablet was Fickian diffusion. This typical behaviour was commonly observed in diffusion controlled drug delivery systems [23].

In our experiments the result of 'n' clearly indicates that the diffusion is the dominant mechanism of drug release from these waxes/fat microspheres (F1, F2,F3 &F4). Diffusion helps to transport the drug from waxes/fat into the *in vitro* study fluid, as gradient varies, the drug is released and the distance for diffusion increases. From this, it was noticed that drug diffuses at a slower rate as the distance for diffusion increases. This is a good agreement with literature findings[24]. The obtained correlation coefficient, $R^2$ for the OZ loaded pellets lies in the range of 0.979 – 0.998. The same result was noticed for Olanex®– 10 mg tablet (0.997). The obtained value of $t_{50\%}$ for all waxes/fat microspheres ( F1, F2, F3 &F4) lies in the range of 4.56 to 5.23 h and 5.313 h was noticed for Olanex®– 10 mg tablet.

The drug release from the optimized formulation F2 was compared with oral formulation Olanex®– 10 mg tablet. The plot of the cumulative percent drug release as a function of time for formulation F2 and Olanex®– 10 mg tablet is shown in Fig 5. From the figure, it is evident that the drug release was controlled from formulation F2 than the commercially available product Olanex®– 10 mg tablet. Differential factor ($f_1$) and similarity ($f_2$) factor was calculated from dissolution profile and the results were compared to the formulation, F5 and Olanex®– 10 mg tablet. The differential factor ($f_1$) and similarity factor ($f_2$) obtained from dissolution profile indicates that the formulation F2 (8.76 & 9.21) and Olanex®– 10 mg tablet (76.124 & 77.02) were similar.

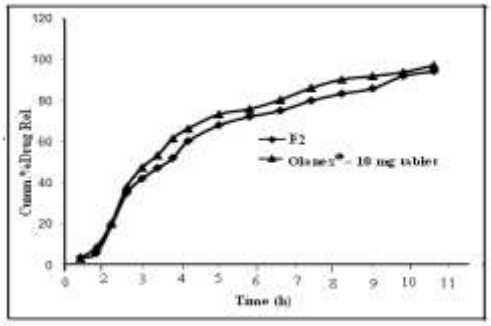


**Figure 5. Cumulative % release of OZ from microsphere F2 & Olanex® in the intestinal environment against time F2 (—♦—) and Olanex®– 10 mg tablet ( ▲ )**

The optimized formulation F2 was subjected for accelerated stability studies. Stability studies were carried out 25$^0$C/ 60% RH, 30 $^0$C/ 65% RH & 40$^0$C/ 75 % relative humidity ( RH) for a period of 90 d (Table 4). It was observed that, no significant change in the drug content from the pellets was observed. It is evident from the table that, formulations F5 exhibited good stability during investigation period, which indicates the drug was in stable form.

**Conclusion**

The present study reports the development of OZ loaded reproducible waxes/fat microspheres could be prepared for intestinal release of OZ using meltable dispersion emulsified method without affecting the chemical nature of the drug. The method is quite esy, simple, rapid, economical and does not imply the use of toxic organic solvents. The drug release from the waxes/fat microspheres was found sufficient for oral delivery and the drug release profile was significantly affected by the properties of waxes/fat used in the preparation of microspheres. These results demonstrate the potential use of waxes/fat for the fabrication of controlled delivery devices for many water insoluble drugs.

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**Table 1. Micromeritic properties of the drug loaded wax/fat microspheres**

| Formulation | Average size (μm) | Yield (%) | Angle of repose ($\theta^0$) | % Compressibility index | Tapped density ($g/cm^3$) |
|---|---|---|---|---|---|
| $F_1$ (OZ :BW – 1:3) | 320 | 84.54 | 26.31 | 10.45 | 0.43 |
| $F_2$ (OZ:CSA – 1:3) | 330 | 90.78 | 27.12 | 12.34 | 0.46 |
| $F_3$ (OZ:SP – 1:3) | 323 | 86.24 | 27.98 | 11.54 | 0.48 |
| $F_4$ (OZ:CA – 1:3) | 312 | 88.32 | 24.89 | 11.98 | 0.44 |

Values shown in the table mean percent of 3 batches (n = 3).
*OZ = Olanzapine,F1= OZ& Beeswa, F2 = OZ & Cetostearyl alcohol, F3 = OZ & Spermaceti, F4 = OZ & Cetyl alcohol*

**Table 2. Drug loading properties of wax microspheres**

| Formulation | Drug loading ( mg) | Encapsulation efficiency (%) |
|---|---|---|
| $F_1$ | 12.28 | 81.32 |
| $F_2$ | 13.89 | 83.93 |
| $F_3$ | 12.78 | 82.52 |
| $F_4$ | 12.12 | 81.10 |

Values shown in the table mean percent of 3 batches ( n=3)

**Table 3. Stability studies for drug content of formulation F2**

| Stability condition | Sampling ( days) | Drug content ( mg) |
|---|---|---|
| $25^0$C / 60% RH | 15 | 99.12 |
| | 45 | 99.07 |
| | 90 | 99.02 |
| 30 $^0$C/ 65% RH | 15 | 99.09 |
| | 45 | 98.98 |
| | 90 | 98.91 |
| 40 $^0$C / 75% RH | 15 | 99.01 |
| | 45 | 98.86 |
| | 90 | 98.84 |

Values shown in the table mean percent of 3 batches ( n=3)

RH – Relative humidity