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Encapsulation of Clozapine into Beeswax Microspheres: Preparation, Characterization and Release Kinetics

D.V. Gowda, Vikas Kumar Gupta*, Mohammed Shuaib Khan, Afifa Bathool

Department of Pharmaceutics, JSS College of Pharmacy, JSS University, S. S. Nagar, Mysore-570015,India.

Corres.author: guptavikas88@gmail.com
Ph. no.: 0821-2548353, Fax: 0821-2548359

Abstract: The objective of the present study was to minimise the unwanted side effects of Clozapine (CZ) drug by kinetic control of drug release, it was entrapped into gastro resistant, biodegradable waxes such as beeswax (BW) 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 92.4%. The 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 95.0% of the isolated spherical microspheres were in the particle size range of 315-328 µm which were further confirmed by scanning electron microscopy (SEM) photographs. The drug loaded in microspheres was stable and compatible, 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 Syclop®. 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.
Key words: Bees wax microspheres, Clozapine, controlled release, kinetic control.

INTRODUCTION

Clozapine [8-chloro-11-(4-methylpiperazin-1-yl)-5*H*-dibenzo[*b*,*e*][1,4]diazepine] is a potential antipsychotic agent used in chemotherapy[1]. It is one of the most commonly used atypical antipsychotics, and is also used for treatment of treatment resistant schizophrenia. To achieve a high level of safety and effectiveness in pharmacotherapy, quality requirements of active substances are growing [2,3]. The starting dose of CZ is 12.5 mg orally once or twice a day. It is practically insoluble in water, having only < 27% oral bioavailability[4]. CZ undergoes extensive first pass metabolism. Dosage adjustments may be needed based upon individual patient characteristics.

The use of clozapine is associated with side effects: extreme constipation, night- time drooling, muscle stiffness, sedation, tremors, orthostasis, hyperglycemia, and weight gain. The risks of extrapyramidal symptoms such as tardive dyskinesia are much less with clozapine when compared to the typical antipsychotics. Clozapine also carries eleven black box warnings for agranulocytosis, CNS depression, leukopenia, neutropenia, seizure disorder, bone marrow suppression, dementia, hypotension, myocarditis, orthostatic hypotension and seizures. To achieve maximum therapeutic effect with a low risk of adverse effects, controlled released preparations are preferred[5,6]. 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[7]. Delivering the drug in the intestinal milieu from wax microspheres could be manipulated by suitable coating techniques[8]. The chief characteristics of enteric coating are their impermeability to gastric juices but susceptibility to intestinal juices[9,10]. Some schizophrenic patients hide a conventional tablet under their tongue to avoid its daily dose of an atypical antipsychotic. To overcome this problem an attempt was made to formulate and evaluate controlled release dosage forms of CZ. Wax microspheres were made to improve the solubility of CZ and to enhance dissolution rate of CZ. It may enhance the pregastric absorption of CZ.

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[9,10]. The objectives of the present study are to formulate, characterize and study the *in vitro* drug release from wax microspheres loaded with CZ. The pattern of drug release from the wax microspheres is compared with that of the commercially available oral formulation Syclop® 25 mg tablet.

MATERIALS AND METHODS

MATERIALS

Clozapine was obtained as a gift sample from Micro Labs Ltd, Bangalore, India. Beeswax, 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 BW was melted separately in china dish using water bath. Drug (3 gm) previously passed through sieve no.100 was dispersed in the melted wax mass and stirred to obtain a homogeneous melt. These individual mixtures were poured into 200 ml of pH 10.9 Ammonia buffer solution (to minimize the solubility of drug), which was previously heated to a temperature higher than melting point of wax (>+ 5°). Tween 80 (1.8 % w/w) was added to the mixture. The whole mixture was mechanically stirred at 900 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 900 rpm at a higher temperature (>+ 5°) of the melting point of wax 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 microspheres (magnification 45 X) were taken on a black paper using Camera Lucida, (Model-Prism type, Rolex, India) and circulatory factor was calculated[16]. The sphericity of microspheres was calculated using the equation,

$$S = p^2 / (12.56 \times A) \quad \ldots. \quad (1)$$

where A is area (cm^2) and p is perimeter (cm)

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 - 4000CM^{-1} to drug excipient interactions if any.

POWDER X-RAY DIFFRACTION PATTERNS (pXRD)

Powder X-ray diffraction (pXRD) patterns were obtained using an X-ray diffractometer 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. pXRD diffraction patterns were recorded for pure CZ and drug loaded wax microsphere.

ESTIMATION OF DRUG LOADING

Drug incorporated wax microspheres of each batch was selected and powdered in a mortar. 100 mg of drug loaded wax 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 225 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 (CZ 25 mg equivalent to a commercial preparation – Syclop® 25 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.

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 15th, 45th and 90th day, checked at regular intervals for changes in physical appearance. Drug content was estimated UV spectrophotometrically at 225 nm.

RESULTS AND DISCUSSIONS

Evidence have shown in the recent years that waxy materials have the physical properties and behaviour suitable to prepare gastro resistant, biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen [11,12]. 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 was obtained at pH 9.2. When pH value changes from 9.2 to 7.0, the percent of drug loading reduced from 10.14 to 1.8 %, 11.32 to 1.6 % , 13.21 to 2.8 % , 10.02 to 1.5 % and 9.32 to 1.6 % for F_1, F_2, F_3, F_4 and F_5 formulations.

In the present study, it was found that 150 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, 150 ml of aqueous phase was used.

Incorporation of CZ into BW microspheres required the addition of tween 80 as a surfactant, 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 was a 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 1.3 to 2.1 % (w/w) of the total formulation were tested. Discrete microspheres with good flow properties using an optimum concentration of surfactant 1.9 % w/w (tween 80) were used. Concentrations of tween 80 ranging from 1.1 to 1.8 % w/w failed to produce reproducible microspheres. The resultant wax microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres. A similar surfactant concentration was reported for carnauba wax and bees wax microspheres prepared by meltable dispersion method [8, 9].

Temperature of the aqueous phase was maintained at 5^0 C higher than the melting point of the BW 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 wax flakes were produced.

In the present study, to produce the spherical discrete microspheres, an optimum drug to wax 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 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 wax microspheres were in the size range of 114 to 500 µm and 56.28 to 61.25 % were of size fraction 245 µm. It was observed that the average size of the microspheres ranged between 315 to 328µm presented in Table 2 and high molecular weight beeswax produced little big sized microsphere[10]. The important factor that influences the size distribution of microspheres is the optimum stirring speed and stirring time. A stirring speed of 900 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 900 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 900 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 900 rpm), there was a decrease in the recovery yield of microspheres. When the stirring time lower than 4 min, some amount of melted material adhered to the sides of the beaker during the cooling process, resulted in lower recovery of yield.

Table 1. Drug and wax ratio for the prepared microspheres formulations

Formulation	Drug	Bees wax
F_1	1.0	2.7
F_2	1.0	2.9
F_3	1.0	3.0
F_4	2.0	3.0
F_5	2.0	3.2

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 [12]. 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 0.41 g/cm^3 to 0.47 g/cm^3. The results of % compressibility index ranges from 10.65 % to 12.36 %, suggests good flow characteristics of the microspheres [Table 2]. The better flow property indicates reasonable and good flow potential of prepared microspheres.

Table 2. Micromeritic properties of the drug loaded wax microspheres

Formulation	**Average size (µm)**	**Yield (%)**	**Angle of repose (θ^0)**	**% Compressibility index**	**Tapped density (g/cm^3)**	**Physical appearance**
F_1	318	86.25	25.69	11.31	0.41	Free flowing
F_2	322	92.57	26.15	10.65	0.44	Free flowing
F_3	325	84.22	28.53	12.36	0.47	Free flowing
F_4	328	91.24	26.33	11.72	0.42	aggregate
F_5	315	88.62	24.48	11.44	0.46	aggregate

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

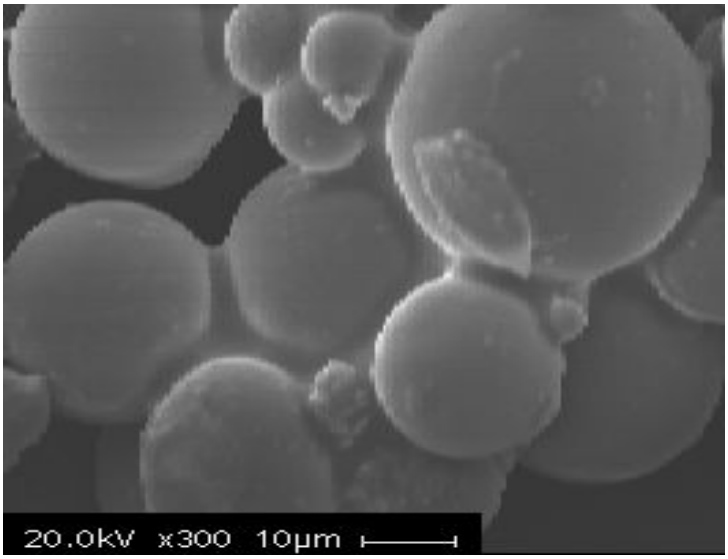

Figure 1. SEM microphotographs of wax microspheres loaded with clozapine showing surface dents and spherical in nature

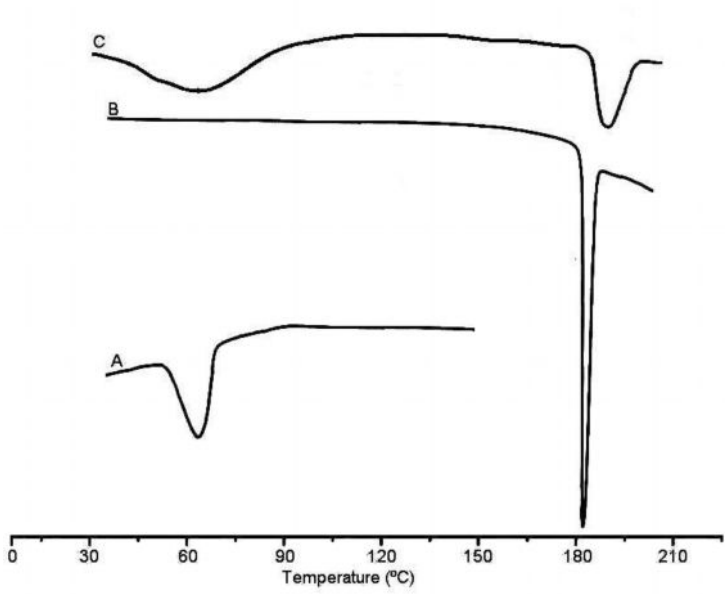

Figure 2. DSC thermograms of beeswax, pure clozapine and clozapine loaded wax Microspheres. [A = beeswax, B = pure clozapine, C = clozapine loaded wax microspheres (F_3)]

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. Photomicrograph reveals the absence of drug crystals on the surface of microsphere, indicating uniform distribution of the drug within the microspheres. The rate of solvent removal from the microspheres exerts an influence on the morphology of the final product[13]. 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, drug-loaded microspheres have shown sharp endothermic peaks. CZ exhibits a sharp endothermic peak at 182.35° C presented in fig. 2. It was observed that presence of the endothermic peak of the drug at 182.62° C in the drug loaded wax microspheres (F_3). The peak intensity corresponding to the melting of CZ decreased in the thermograms of CZ loaded wax

microspheres. These results indicate that only a small fraction of the drug substance existed in the crystalline state. Reduction in the melting point and enthalpy of the melting endotherm was observed when the wax was formulated as microspheres. Incorporation of CZ inside the wax matrix results in decrease in the melting point of the wax in the final formulation (F_3). Small particle size of wax microspheres leads to high surface energy, which creates an energetically suboptimal state causing a decrease in the melting point[14].

From the FTIR studies, the characteristic bands for important functional group of pure drug empty microspheres and drug-loaded microspheres were identified. FTIR spectra showed that the characteristics bands of CZ were not altered after successful encapsulation without any change in their position, indicating no chemical interactions between the drug and wax used. Compared the IR spectra at 3293 cm^{-1} due to NH stretching, 2968 cm^{-1} due to aliphatic C-H stretching, 1462 cm^{-1} aromatic C = C stretching, 1551cm^{-1} C = N stretching, and 820 cm^{-1} due to C – cl stretching. A comparison and interpretation of this region in our spectra agrees with their conclusions[14].

The XRD spectra recorded for the pure clozapine (A), CZ loaded wax microspheres (B) are presented in Fig. 4. These studies are useful to investigate crystallinity of the drug in loaded wax microspheres. Clozapine has shown characteristic intense peaks between 2θº of 9.52 and 11.04 due to the presence of clozapine crystals. However, these peaks were observed in CZ loaded wax microspheres. Beeswax had a principal peak at around the same 2θ° (19.267). The principal peak of CZ (9.45, 11.13) was present in CZ loaded wax microspheres. Furthermore, the principal peak of the wax did not shift, but had a reduced intensity[15].

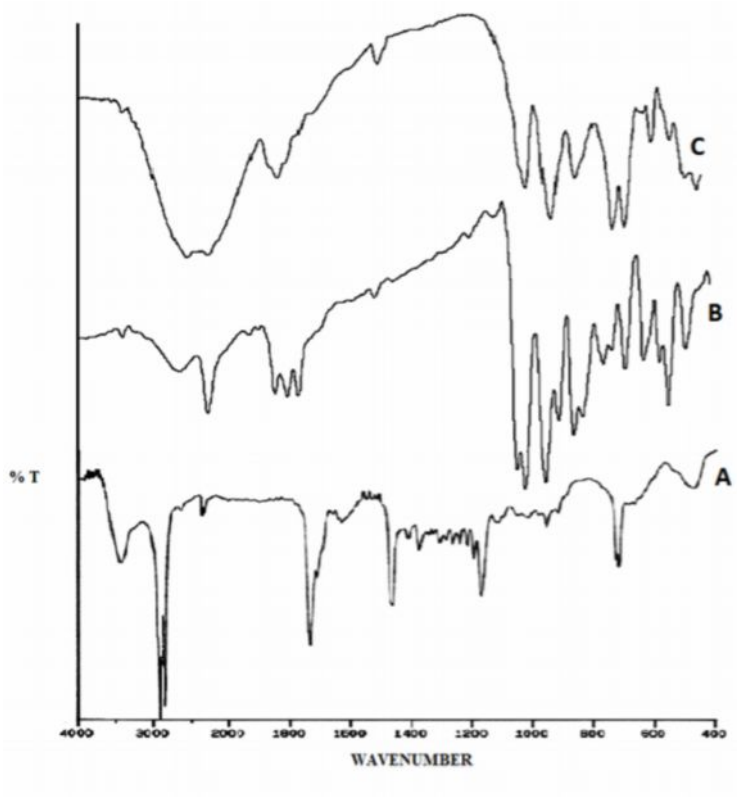

Figure 3. FTIR spectra of BW (peak A), CZ (peak B) and CZ loaded wax microspheres (peak C - F_3). [BW = beeswax, CZ = pure clozapine, F_3 = clozapine loaded microspheres]

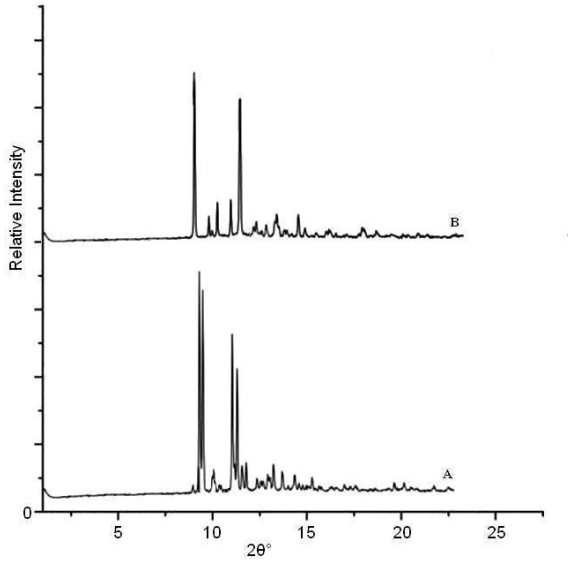

Figure 4. X-ray diffraction pattern of CZ (peak a) and CZ loaded wax microspheres (peak b - F_3). [Peak A = clozapine, peak B = clozapine loaded wax microspheres (F_3)]

Table 3. Drug loading properties of wax microspheres

Formulation	Drug loading (mg)	Encapsulation efficiency (%)
F_1	14.54	87.22
F_2	13.85	89.62
F_3	15.73	91.37
F_4	13.29	83.15
F_5	13.95	82.19

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

Table 4. *In vitro* release kinetic parameters for beeswax microspheres

Formulation	n	Higuchi (R^2) at 0.1NHCl	Higuchi (R^2) at pH 7.4 PB
F_1	0.43	0.9922	0.9954
F_2	0.46	0.9906	0.9974
F_3	0.47	0.9915	0.9985
F_4	0.42	0.9969	0.9953
F_5	0.44	0.9933	0.9968

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

Table 5. Stability studies for drug content of formulation F_3

Stability condition	Sampling (d)	Drug content (mg)
25 ^{0}C / % RH	15	99.18
	45	99.12
	90	99.07
30 ^{0}C / % RH	15	99.15
	45	99.04
	90	98.92
40 ^{0}C / % RH	15	99.08
	45	99.02
	90	98.88

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

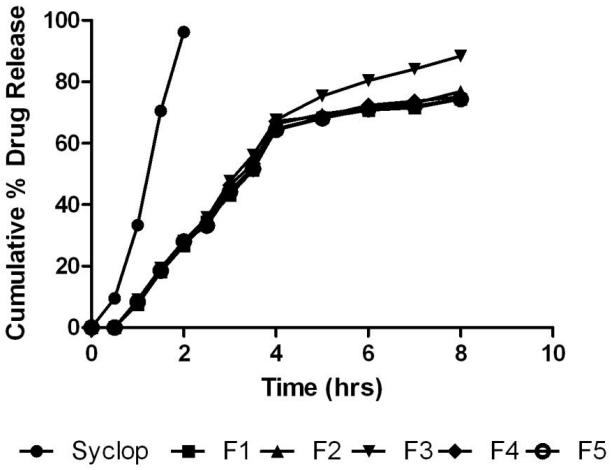

Figure 5. Cummulative % release of clozapine from wax microspheres and Syclop® in the gastric and intestinal environment against time

The percent of drug loading in the formulations was found to be in the range of 13.29 % to 15.73 %. It was low in the formulation F_4 and more in F_3. The percent of encapsulation efficiency was found to be 91.37% in formulation F_3 and the results are presented in Table 3.

From the release studies it was observed that, there is no significant release of drug at gastric pH from wax microspheres. *In vitro* drug release from Syclop® (92.23% in 2 h at gastric environment) was faster than F_3 (85.47 % in 8th h), in the intestinal environment as shown in Fig 5. Drug was released in a biphasic manner consisting of initial fast release followed by a slow release in intestinal pH from the wax microspheres[10,11,12]. The decreased *in vitro* drug release from wax microspheres might be due to more hydrophobicity and influence of molecular weight of wax. The *in vitro* drug release was considerably retarded from the wax microspheres when compared Syclop®. 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 wax microspheres was fickian diffusion presented in table 4. After an initial burst effect, the subsequent release of drug from microspheres was slow, and the influence of molecular weight (MW) was observed. The release profiles of the wax microspheres best fit into the Higuchi equation. The Higuchi equation describes the diffusion of drug from homogenous wax matrix systems. The drug release from a wax matrix system is said to follow Higuchi's release kinetics if the amount of drug released is directly proportional to the square root of time. The slopes obtained from the above plot are proportional to an apparent diffusion coefficient. Excluding the burst effect by omitting the early time data points (time points up to 2 hours), linear fits were obtained indicating that release was diffusional. All the wax microspheres exhibited initial burst release followed

by controlled release. The initial *in vitro* burst release is probably due to surface accumulated drug on wax microspheres. The controlled release is probably due to diffusion of drug from the wax matrix. GTS, the most lipophilic beeswax in this study, had the highest controlled release effect.

Stability studies for the formulation F_3 were performed to ascertain whether the drug undergo any change or degradation during its shelf life. These samples were checked for changes in physical appearance and drug content at regular intervals. The obtained results of the stability studies are given in Table 5. From the results, the drug was stable in the prepared formulations for the study period.

CONCLUSION

The method is quite simple, rapid, economical and does not imply the use of toxic organic solvents. The drug release from the bees wax microspheres was found sufficient for oral delivery and the drug release profile was significantly affected by the properties of wax used in the preparation of microspheres. These results demonstrate the potential use of wax for the fabrication of controlled delivery devices for many water soluble drugs.

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