Current Pharma Research
ISSN-2230-7842
CODEN-CPRUE6
www.jcpronline.in/

Review Article

Formulation Design and Pharmacodynamic evaluation of Alginate microspheres of Olanzepine.

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Received 01 June 2018; received in revised form 22 June 2018; accepted 22 June 2018

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ABSTRACT
In the present study, alginate based microspheres of Olanzepine was prepared by ionotropic external gelation technique utilizing calcium chloride as a cross linking agent to enhance bioavailability and residence time. The prepared microspheres are discrete, spherical and free flowing which was characterized by entrapment efficiency, particle size, in-vitro release behavior, scanning electron microscopy, in-vivo study etc; Optimized formulation was followed zero-order release kinetic. In-vivo evaluation of antidepressant activity of Olanzapine formulation showed that duration of immobility time was significantly decreased as compare to control and standard marked SR formulation. The optimized formulation was mucoadhesive in nature. Stability studies was carried out for F9 at a temperature of 40±2°C/ RH 75±5% formulation revealed that the drug behavior was within permissible limits.

KEYWORDS
Inotropic gelation, Microsphere, Gastro retentive delivery, Mucoadhesive.

1. INTRODUCTION

Oral controlled drug delivery system such as microsphere drug delivery system used to prolong the residence time, maintain therapeutically effective plasma drug concentration, reducing the dosing frequency and to minimize fluctuations in the plasma drug concentration at the steady state. Formed microspheres become adhesive on hydration, and used for localizing the drugs to a particular target site of gastrointestinal tract for prolong periods of time. It is easy for administration, no patient compliances and flexibility in the formulation. Mucoadhesive microspheres have advantages like efficient absorption, enhanced bioavailability of the drugs, maximum utilization of drugs, and better patience compliance.[1]

Olanzepine is an atypical antipsychotic drug used in the treatment of schizophrenia. Olanzepine is poorly soluble belongs to BCS class II. It is practically insoluble in water, having only 60% oral bioavailability. Olanzapine undergoes extensive first pass metabolism. In this regard our main focus of this research is to prepare sustain microspheres of Olanzepine which provides slow release in gastrointestinal tract and also assures the presence of dosage form at the site of absorption. The drug has a moderate elimination half-life implying that once daily therapy is adequate for treatment of schizophrenic conditions[2, 3] Therefore, a drug delivery such as "mucoadhesive microsphere", has been applied. Hence, the objective of the present work was to formulate the mucoadhesive microsphere of Olanzepine to improve residence of dosage form in GIT, reduced dosing frequency and enhance bioavailability in the treatment of schizophrenia.

2. MATERIALS AND METHODS

2.1. Materials

Olanzapine was obtained from Enaltec Lab Private Ltd, Mumbai, India. Sodium alginate gift sample from Loba chemical Mumbai and Calcium chloride was purchased from S.B. Fine chemicals Ltd, Mumbai.

2.2. Method: Preparation of microsphere by ionic gelation method

The polymeric solution was soaked overnight by dissolving sodium alginate (3% w/v) in distilled water. Drug (1gm) was dispersed in the polymeric solution. The prepared drug-polymer solution was added drop wise by a 20 gauge hypodermic needle in to 100 ml of 5%w/v of crosslinking agent (Calcium chloride), and stirred continuously at 100rpm for 2 hrs. for complete reaction

2.3. Optimization of microsphere formulation

Table 1. Formula and Composition with Process Variables.

Formulation code	Amount of Olanzapine	Conc. of sod. alginate	Conc. of ca. chloride)
F1	1gm	1%	1%
F2	1gm.	1%	3%
F3	1gm.	1%	5%
F4	1gm	3%	1%
F5	1gm	3%	3%
F6	1gm	3%	5%

F7	1gm	5%	1%
F8	1gm	5%	3%
F9	1gm	5%	5%

2.4. Characterization of Microspheres

2.4.1. Encapsulation efficiency

The drug entrapment efficiency was determined by dissolving weighed amount (100 mg) of microspheres and suspended into 100 ml 0.1 N Hcl for 2 hrs. Solution was filtered and assayed at 227 nm.

2.4.2. Shape and Surface Morphology

The external morphology of the microspheres was studied by scanning electron microscopy. The dried microspheres were mounted on slab using carbon plate and subjected to acceleration voltage of 20 kv with secondary electron image to observe surface picture.[4]

2.4.3. Fourier transforms infrared spectroscopy (FTIR) studies

FTIR spectroscopy was carried out to characterize the possible interaction between drug and excipient. FTIR of pure Olanzapine and polymer were determined to check the intactness of the drug in the polymer mixture using FTIR – spectrophotometer.[5]

2.4.4. Differential scanning calorimeter (DSC) studies

DSC was performed for pure Olanzapine and Olanzapine microspheres using a DSC Perkin Elmer apparatus. Sample heated from 25°C -300°Cat a heating rate of 10°C/minutes in nitrogen atmospheres.[6]

2.5. In-Vitro dissolution

In-vitro drug release studies from the microspheres was studied in phosphate buffer of pH 7.4 (900 ml) using a dissolution rate test apparatus with a rotating paddle stirrer at 50 rpm and 37 ± 1°C. A sample of microspheres equivalent to 10 mg of Olanzepine was used in each test. Samples assayed at 227 nm for Olanzepine content using a Shimadzu UV- 1700 double beam spectrophotometer and percentage drug release was calculated.[7]

2.6. In-vivo study

The approval of the Institutional Animal Ethics Committee was obtained before starting the study. The registration number, approval number and date is RDCOP/ IAEC/ Approval / 2016 – 17/ 01. Animal experiments are conducted in full compliance in conformity with the CPCSEA guidelines. *In- vivo* antidepressant study of such as forced swimming test and tail suspension test were carried out.

a. Mice forced swim test:

Test was performed on mice. Mice were individually placed in a acrylic cylinder ((height: 40 cm; diameter: 18 cm)) filled upto 20 cm with water at 25±2°C, and were forced to swim for 15 minutes. During this 15 minutes session, duration of immobility was observed and recorded. [8,9]

b. Mice tail suspension test:

Test animals were hanged in such way that the mice were individually suspended by the tail to horizontal bar (distance from floor 50 cm) using adhesive tape (distance from tip of tail was

approximately 1 cm.). Immobility time was recorded during a 6 min period .Test performed in replicate to get confirmed result.[10]

2.7. Stability study

Stability studies were carried out for Olanzepine microsphere as per ICH guidelines. The best mucoadhesive microspheres formulation (F9) was sealed in high-density polyethylene bottles and stored at 25±2°C/60±5%, 40±2°C/75±5% relative humidity (RH) for 90 days. The samples (F9) were evaluated.[11]

3. RESULT AND DISCUSSION

3^2Factorial design carried out for factor influencing response variable. It has been observed that concentration of sodium alginate (Polymer) and concentration of calcium chloride (crosslinking agent) had higher influence. Total 9 formulations were prepared as per experimental design

3.1. S.E.M. analysis

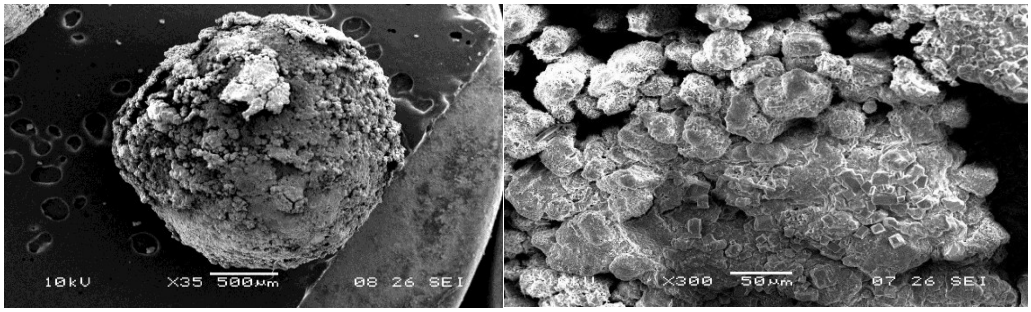

Figure 1. S.E.M. of formulation

SEM image of drug is dispersed in the matrix.SEM image of drug loaded microsphere are shown in fig.1.The microsphere were found nearly spherical shape with rough outer surface due to entanglement of polymer chain which is responsible for sustained release property.

3.2. Encapsulation efficiency

It was observed that conc. of these factors have impact on percentage release. The percentage yield of different batches was found to be 71.5%to 86.1% respectively. The yield was good indicating that sodium alginate undergo gelation with calcium chloride to form microsphere.

Table 2. Process yield.

Formulation code	Process yield (%)
F1	71.5 ± 0.2160
F2	72.5± 0.2943
F3	72.67± 0.05099
F4	78.2± 0.08165
F5	78.4± 0.3559
F6	78.9± 0.3558
F7	85.2± 0.08165
F8	85.6± 0.3559
F9	86.1± 0.1441

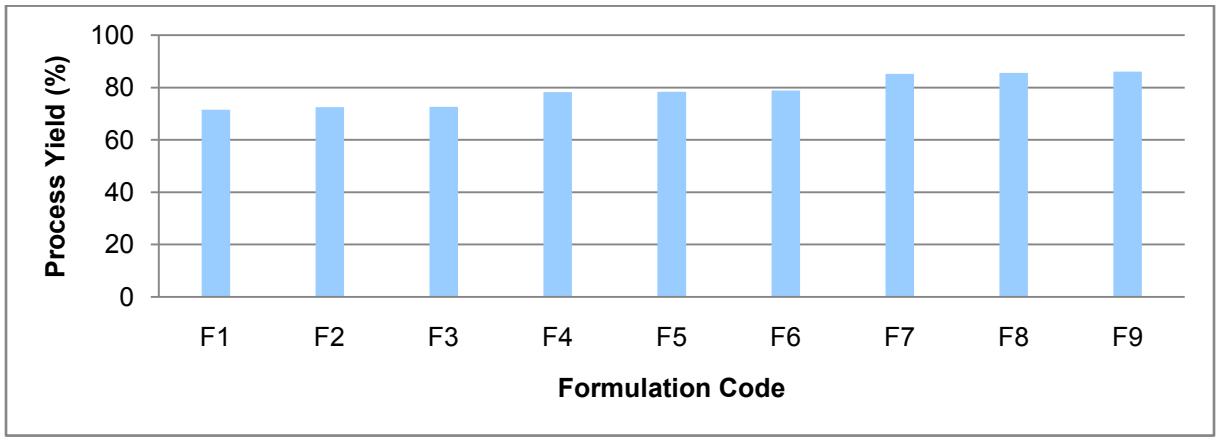

Figure 2. Encapsulation efficiency

3.3. Fourier transforms infrared spectroscopy (FTIR) studies

It was observed that Olanzapine showed characteristic peak at 3337 cm^{-1} for – NH group whereas sodium alginate showed –CO group at 1670 cm^{-1}, –OH group at 3100 cm^{-1} and –NH group at 3563 cm^{-1} However shift in –CO group peak of polymer and – NH group of Olanzapine to 1696 cm^{-1} and 3563 cm^{-1}suggested possibility of H – bonding between drug and polymer.

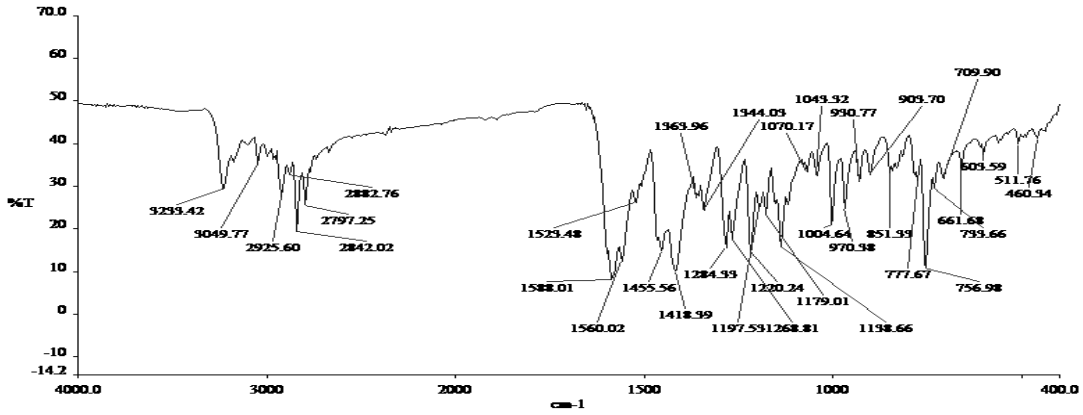

Figure 3. F.T.I.R. of pure Olanzepine

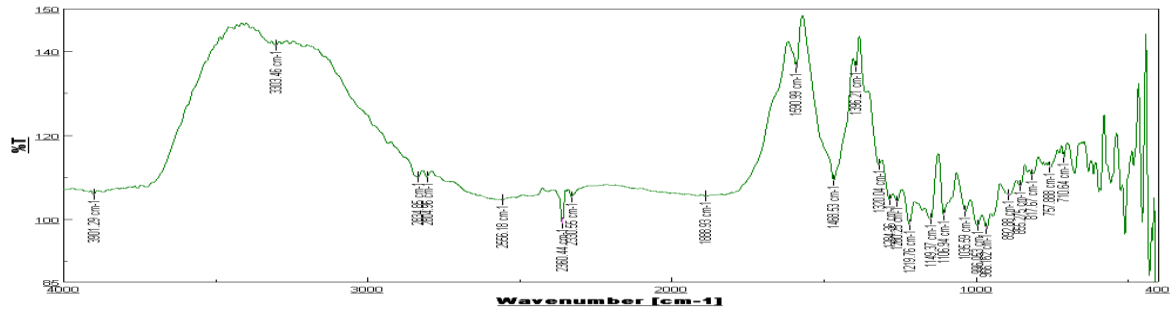

Figure 4. FTIR of Olanzepine microsphere

3.4. Differential scanning calorimeter (DSC) studies

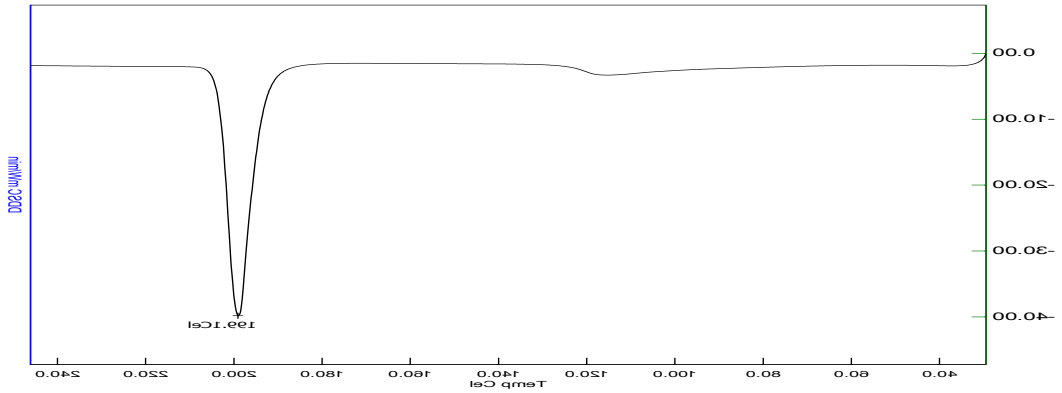

Figure 5. Differential scanning calorimeter thermograms Pure Olanzepine

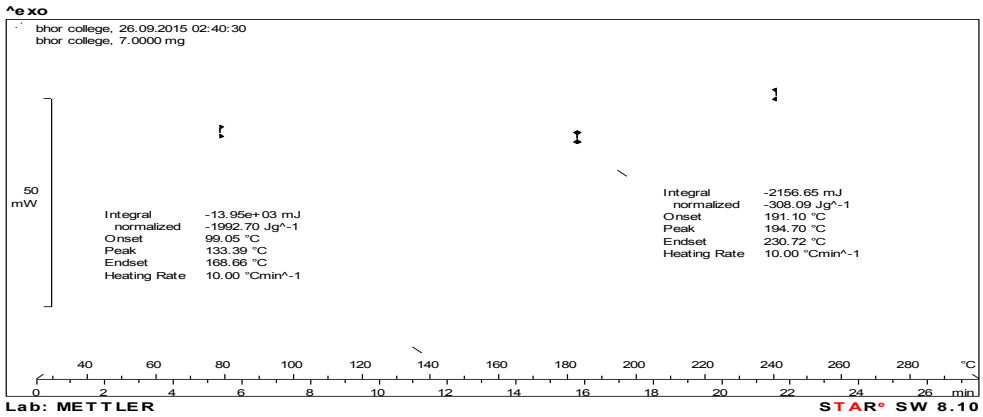

Figure 6. D.S.C. of Olanzepine microsphere

It was reported that Olanzepine has melting point of 199.1ºC whereas formulation showed 2 melting endotherm at DSC thermogram at 133.9ºC and at 194.70ºC correspond to melting point of polymer and drug indicating that there is no interaction between drug and polymer.

Table 3: %*in-vitro* drug release

Formulation code	***In-vitro* Dissolution (%)**
F1	92.3±0.294
F2	85.3±0.141
F3	63.4±0.355
F4	74.8±0.294
F5	64.7±0.432
F6	39.1±0.0816
F7	31.3±0.294
F8	29.5±0.2943
F9	32.4±0.294

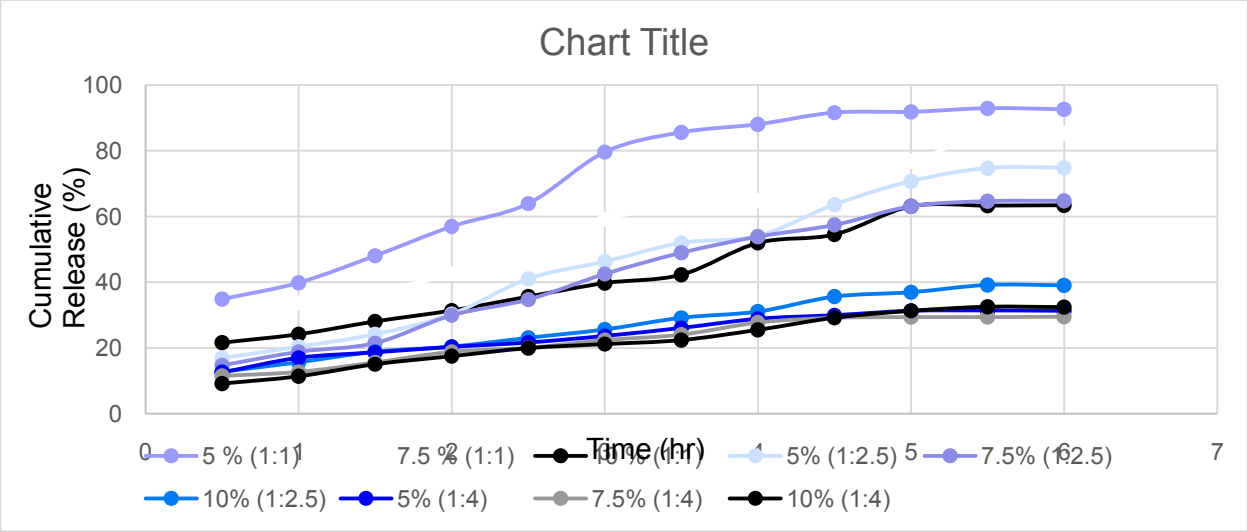

Figure 7. Drug release of Olanzepine microsphere

In-vitro release study showed that formulation was found to be between 32.4% to 92.3% respectively. It has been found that that as the concentration of the polymer increases drug content increases and as the conc. of crosslinking agent decreases.

3.5. In-vivo antidepressant study

In-vivo study such as forced swim test and tail suspension test showed antidepressant activity of Olanzepine microsphere compared with marketed formulation in test animal.

Table 4: Forced swimming test

S. No.	Control	Olanzepine SR	Olanzepine Microsphere
1	134	74	71
2	103	96	45
3	86	56	66
4	107	76	77
5	123	57	52
Mean	110.6	71.8	62.2
std. deviation	18.56	16.41	13.33
Std. Error	8.298	7.338	5.962

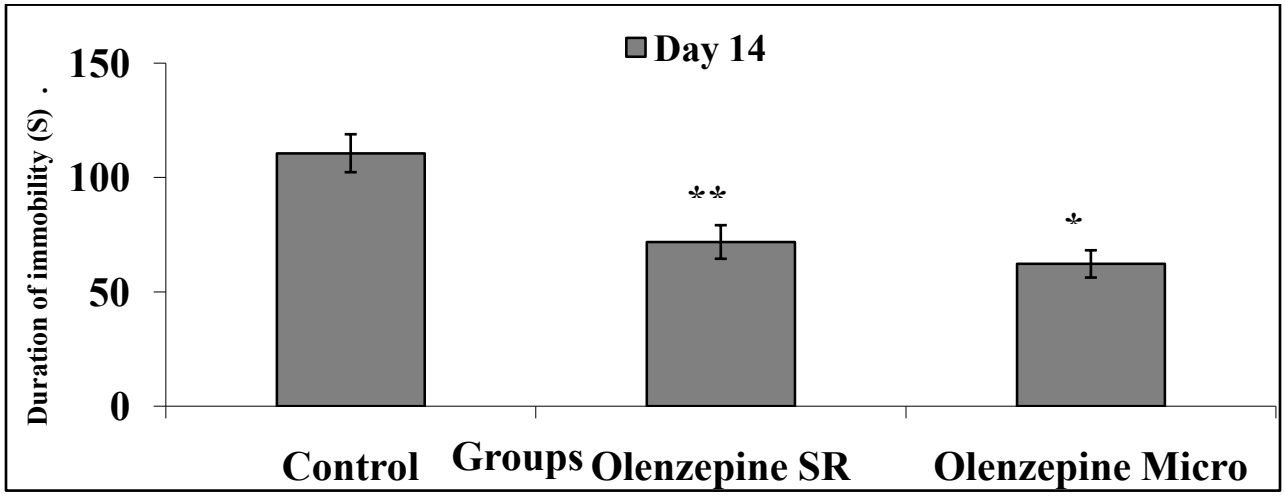

Figure 8. Forced swim test

Table 5: Tail suspension test

S. No.	Control	Olanzepine SR	Olanzepine Microsphere
1	93	77	55
2	68	73	51
3	86	67	49
4	101	43	33
5	112	56	21
6	95	39	23
Mean	92.5	59.17	38.67
std. deviation	14.84	15.8	14.94
Std. Error	6.059	6.452	6.097

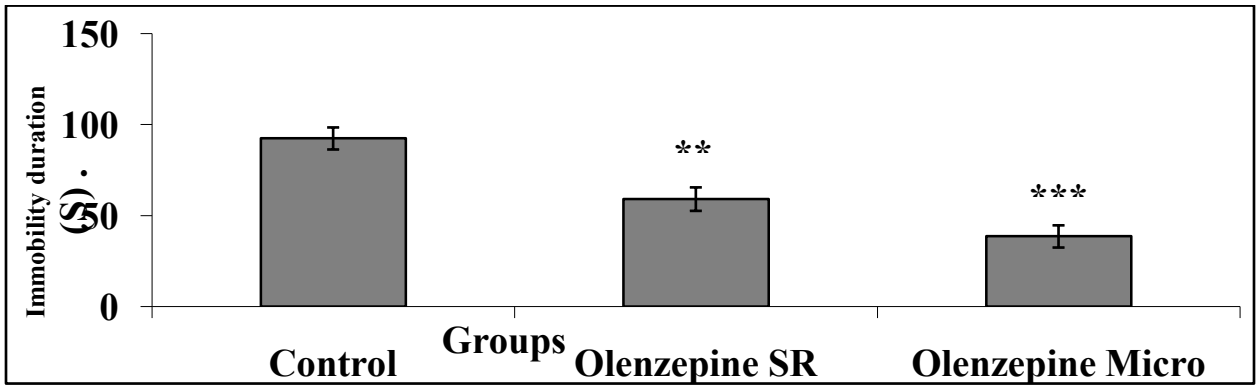

Figure 9. Tail suspension test

3.6. Stability study

The prepared formulation was found to be stable under accelerated stability study for three months. This confirmed that Olanzepine microsphere dosage form for effective delivery of Olanzepine.

Table 6: Stability study

Sr. no	Duration	Drug Content (%)	(%) *in-vitro*
0	0 month	82.00	30
1	1 month	82.34	30.2
2	2 month	82.45	30.21
3	3 month	82.89	30.33

4. CONCLUSION

The microsphere of Olanzepine was prepared by ionic gelation method. Particle size and shape of microsphere were examined of optimised formulation was spherical with no surface irregularity. FTIR spectrum was observed that there were no major shifts in main peaks of drug. This indicates that there was no compatibility issues of drug with formulation polymers used.The maximum percentage yield was found of F9 batch and was noted to be 86.1 % over 6 hr. It was found that DSC curves obtained for showed a sharp melting endotherm peak at.199. The initial drug release of Olanzapine microsphere at 1hr is 9.2% and then found 92.23% at the end of 6 h. It was found that drug release rate decreased as the concentration of sodium alginate increased and also with increased cross-linking time. The stability studies were carried out on optimizes formulation i.e.F9.*In-vivo* evaluation of antidepressant activity of Olanzapine formulation showed that duration of immobility time was significantly decreased as compare to control and standard marked SR formulation.

5. ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacy, R. D. College of Pharmacy Bhor for providing materials and support.

6. ETHICAL ISSUES:

Not applicable.

7. CONFLICT OF INTEREST:

Authors declare no conflict of interest in this study

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