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Research Article

Formulation and Evaluation of Olanzepine Microspheres.

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ABSTRACT

The aim of this research was to formulate and evaluate mucoadhesive microspheres of Olanzapine drug to enhance bioavailability and enhance residence time. Olanzepine mucoadhesive microsphere prepared by using sodium alginate polymer and calcium chloride as a crosslinking agent. .Microsphere were prepared by using ionic gelation method. Microsphere with particle size in the range 656.4µm to 659 µm. were prepared by using ionic gelation method. Olanzapine (OLE) having extensive hepatic first pass metabolism and low bioavailability problem, Olanzapine microspheres were evaluated for mean particle size, the percentage yield, entrapment efficiencies, in-vitro release, *in vitro* mucoadhesive, FTIR, DSC, X-ray diffraction studies and stability study. Formulation F9 microspheres batch was found to be optimized and followed zero-order release kinetic .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 Mucoadhesive microsphere drug delivery systems to prolong the residence time at the site of application or absorption microsphere is useful to maintain therapeutically effective plasma drug concentration levels for a longer duration there by reducing the dosing frequency and to minimize fluctuations in the plasma drug concentration at the steady state by delivering the drug in a controlled and reproducible manner. Mucoadhesive microspheres become adhesive on hydration, and hence can be used for localizing the drugs to a particular target site of gastrointestinal tract (GIT) for prolong periods of time. Moreover, it is easy for administration, no patient compliances and flexibility in the formulation. One of the most feasible approaches for achieving a prolonged and predictable drug delivery in gastrointestinal tract (GIT) is to control gastric resistance time i.e., gastroretentive drug delivery system, which will provide us with new and important therapeutic options. Mucoadhesive microspheres have advantages like efficient absorption, enhanced bioavailability of the drugs, maximum utilization of drugs, much more intimate contact with intestinal cells, better patience compliance and targeting to specific absorption site .[1-3].

Olanzapine 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.

Olanzapine has been shown to selectively bind to central dopamine D2 and serotonin (5-HT2c3receptors and is effective against the negative symptoms of schizophrenia with a lower incidence of extra pyramidal symptoms. A second generation atypical antipsychotic, Olanzapine is extensively metabolized in liver (1st pass metabolism) by the cytochrome P450 CYP1A2. The drug has a moderate elimination half-life implying that once daily therapy is adequate for treatment of schizophrenic conditions[4-6]. 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 Olanzapine 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. Preparation of microsphere

The Microspheres were prepared using an ionic crosslinking technique. The polymeric solution was soaked overnight by dissolving sodium alginate (3% w/v) in distilled water. The Olanzapine (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. The

microspheres were recovered by filtration through a whatmann filter paper and washed 2-3 times with deionized water and air dried. Prepared microspheres were evaluated by different parameters. [7-8]

2.3. Percentage yield

The percentage yield of olanzapine microspheres of various batches were calculated by using the weight of final product after drying with respect to initial total weight of the olanzapine and polymer used for preparation of olanzapine mucoadhesive microspheres .[12]

2.4. Scanning electron microscopy (SEM)

The sample was loaded on copper sample holder and sputter coated with platinum. The external and internal morphology of the microspheres were studied by scanning electron microscopy (SEM) in Pune University (Physics Department).The samples for SEM were mounted on metal stubs prepared.[9]

2.5. Factorial Design

A 3^2 full factorial design was constructed using design expert for mathematical modeling and analysis of responses where the amounts of Polymer(X_1) and crosslinking (X_2) were selected as the independent factors. The levels of the two factors were selected and on the basis of the preliminary studies carried out before implementing the experimental design. A statistical model was used to evaluate the responses which involve polynomial terms.

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1 + b_{22}X_2^2$

Where Y is the dependent variable, b0 is the arithmetic mean response of the 9 runs, and bi is the estimated coefficient for the factor Xi. The main effects (X1 and X2) represent the average result of changing one factor at a time and (X1X2) represent interaction factor[10]

2.6. Drug entrapment efficiency

Weighed amount of Olanzapine microspheres (50mg) was soaked in 10 ml of water. This solution was kept 1 hr. for the complete swelling of microspheres in the water. This solution was filtered and further diluted with methanol to make a conc. of 10 µg/ml solution. The absorbance of the solutions was measured at 227 nm using double beam UV-Visible spectrophotometer and the percentage of drug present in the sample was calculated.[11]

2.7. In-vitro Wash off Test for Microspheres

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test. A 1-cm by 1-cm piece of sheep intestine mucosa was tied onto a glass slide (3-inch by 1- inch) using thread. Microspheres were spread onto the wet, rinsed, tissue specimen, and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 1.2). At the end of 30 minutes, 1 hour, and at hourly intervals up to 2 hours, the number of microspheres still adhering onto the tissue was counted. [12]

2.8. In Vitro dissolution

In-vitro Drug Release Studies Release of Olanzapine 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 olanzapine was used in each test. Samples of dissolution fluid were withdrawn at different time

intervals and were assayed at 227 nm for olanzapine content using a Shimadzu UV- 1700 double beam spectrophotometer (Shimadzu Corporation, Japan). From this percentage drug release was calculated.[13]

2.9. Release kinetic studies

The rate and the mechanism of release of olanzapine from the prepared mucoadhesive microspheres were analyzed by fitting the dissolution data into various kinetic models like zero order; first order, Higuchi's model and coefficient of correlation (r) values were calculated for the linear curves by regression analysis of the above plots.[14-15]

2.10. Fourier transforms infrared spectroscopy (FTIR) studies

Infrared spectra for pure Olanzapine and for the physical mixture of Olanzapine and polymer was determined to check the intactness of the drug in the polymer mixture using FTIR – Spectrophotometer. The samples were analyzed between wave numbers 4000 and 400/cm resolution.

2.11. Differential scanning calorimeter (DSC) studies

The thermal behavior of pure olanzapine and olanzapine microspheres were studied using a DSC Perkin Elmer DSC at a heating rate of 10°C/minutes. Samples were accurately weighed into aluminum pans and then sealed. The measurements were performed at a heating range of 25-300°C under nitrogen atmospheres [16]

2.12. X-ray diffraction study

The crystallinities of olanzapine and Olanzapine loaded mucoadhesive microspheres were evaluated by XRD measurement using an X-ray diffractometer (Brucker*).* Powder X-ray diffraction patterns were recorded by X-ray diffractometer (x-Pert, Philips, UK) using Cu-Ka radiation (1.542A) with a voltage of 40 kV and a current of 35 mA. Samples were scanned from 2° to 50°**.**[17]

2.13. 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 for entrapment efficiency and percentage mucoadhesion.[18]

3. RESULTS AND DISCUSSION

The Olanzepine microsphere were prepared by orifice ionic gelation method. The formula optimization was done by 3^2 factorial design. The significant factors selected were concentration of sodium alginate and crosslinking agent. The dependent variables selected were entrapment efficiency, % mucoadhesion and % drug release. The model was analysed for fitting into appo. Mathematical model and evaluated statistically for ANOVA. The response surface analysis was carried out employing the 3D response surfaces.

3.1. Percentage yield

The percentage yield of microspheres were calculated by using the weight of final product after drying with respect to initial total weight The maximum percentage yield was found of F9 batch and was noted to be 86.1 % among all the batches. The production yields of microspheres

prepared by ionotropic gelation method were found to be between 71.5 % an 86.1 % as shown in table 3.

Table 1: 3^2 full factorial design layout, experimental runs and their combinations:

Sr. No	Batch No	X1	X2
1	F1	-1	-1
2	F2	-1	0
3	F3	-1	1
4	F4	0	-1
5	F5	0	0
6	F6	0	1
7	F7	1	-1
8	F8	1	0
9	F9	1	1

Table 2: Formula and Composition with Process Variables.

Formulation code	Amount of olanzapine (mg.)	Conc. of Sodium Alginate (mg)	Concentration of Calcium Chloride(%w/v)
F1	100	100	5
F2	100	100	7.5
F3	100	100	10
F4	100	250	5
F5	100	250	7.5
F6	100	250	10
F7	100	400	5
F8	100	400	7.5
F9	100	400	10

Table 3: Physicochemical properties of olanzepine mucoadhesive microspheres.

Formulation code	Percentage Yield	Particle size	Drug entrapped
F1	71.5	658.2 µm	8
F2	72.5	658.1 µm	12
F3	72.67	657.2 µm	12.4
F4	78.2	656.4µm	60
F5	78.4	660.9 µm	60
F6	78.9	657.1 µm	64

F7	85.2	659.1 µm	84
F8	85.6	658.1 µm	76
F9	86.1	658 .1 µm	84

3.2. Particle size

The average particle size of olanzapine microspheres ranged from 657.1 µm-659.1 µm. The mean particle size was significantly increases with increasing mucoadhesive polymer concentration.

3.3. Morphology of microspheres

The morphology of the mucoadhesive microspheres of best formulation F9 was examined by SEM. The SEM photographs revealed that ritonavir microspheres were discrete and irregular shape with a rough surface morphology (Fig. 1).

3.4. Entrapment efficiency

The entrapment efficiency of the olanzapine microspheres prepared with sodium alginate polymer. The maximum percentage yield was found of F9 batch and was noted to be 86.1 % among all the batches. Increase in conc. of the sodium alginate and ca. chloride increased the entrapment efficiency of the microspheres.

3.5. Mucoadhesive test

To assess the mucoadhesive property of Olanzapine mucoadhesive microspheres, *in-vitro* wash-off test was carried out for all batches, and the results are shown in Table **4**. Percentage mucoadhesion increased with the increase in concentration of polymer. The rank order of percentage mucoadhesivity of all the microsphere formulations after 8 hrs was found to be as follows F9 > F8 > F7 > F4 > F6> F3 > F5> F2 > F1.

3.6. In-vitro Drug release studies

The ***in vitro*** drug release studies were carried out for olanzapine microspheres in phosphate buffer (pH 7.4). Various microspheres (F1 –F9) showed release of olanzapine over 6 h. The ***invitro*** drug release data of optimized microspheres were evaluated kinetically using various mathematical models.

The initial drug release of olanzapine microsphere at 1hr is 9.2% and then found 92.23% at the end of 6 h.

Table 4: Characterization of olanzepine mucoadhesive microspheres.

Formulation code	Percentage Mucoadhesion:	*Invitro* release
F1	6	92.3
F2	10	85.3
F3	16	63.4
F4	24	74.8
F5	26	64.7
F6	34	39.1

F7	34	31.3
F8	38	29.5
F9	44	32.4

3.7. Release kinetic study

In vitro drug release data fitted into various mathematical models. All the microsphere formulations (F1-F9) followed Higuchi model with regression values ranging from 0.9009 to 0.9873.

Table 5: *Invitro* release kinetics parameters for Olanzapine Microspheres.

Formulation code	**Zero Order Model R^2**	**First-Order Model R^2**	**Higuchi Model R^2**
F1	0.9009	0.862	0.9472
F2	0.9701	0.9621	0.9873
F3	0.9441	0.9759	0.9779
F4	0.9862	0.9462	0.9669
F5	0.9761	0.9246	0.9746
F6	0.9883	0.9597	0.9756
F7	0.9565	0.9086	0.9804
F8	0.9454	0.9071	0.9699
F9	0.9829	0.9333	0.9767

Table 6: ANOVA Output.

Sr. No.	Outcomes	Entrapment Efficiency (%)	% mucoadhesion	%Drug Release
1	F value	251.02	73.890	38.29
2	P value	0.0004	0.0023	0.0087
3	R^2 value	0.9981	0.9922	0.9872
4	Adequate Precision	47.378	22.832	18.403

3.8. FTIR studies and DSC studies

Infrared (IR) spectra of pure drugs sample of olanzepine were compared with IR spectra of olanzepine loaded microspheres, as there was no significant change in the pattern of peaks of pure drug and olanzapine loaded microspheres (Fig 6). Hence, there was no interaction seen in between olanzapine and polymers. The thermal behavior of prepared olanzapine microspheres was studied in comparison with thermo grams of pure olanzapine as shown in (Fig.7) The thermogram of pure olanzapine showed a sharp endothermic peak at 184.45 °C, which

correspond to its melting point. Thus, there was noincompatibility between olanzapine and sodium alginate polymer.

3.9. XRD study

The X-ray diffractogram of olanzepine showed sharp peaks depictinga typical crystalline pattern. Physical mixtures showed less intense peaks, however olanzapine loaded mucoadhesive microspheres showed less intense peaks, however ritonavir loaded mucoadhesive microspheres showed peaks, but of low intensity, revealing that some amount of olanzapine was changed to amorphous form. This diminished peak suggests conversion of drug into amorphous form.

3.10. Stability studies

Stability studies for the optimized microsphere was carried out at a temperature of 40±2°C/ RH 75±5% for a period of 90 days. Formulation were evaluated for physical appearance and drug content. There was no any significant change in physical appearance and drug content during stability studies. Hence, it was concluded that the F9 batch of tablet have good stability during their shelf life.

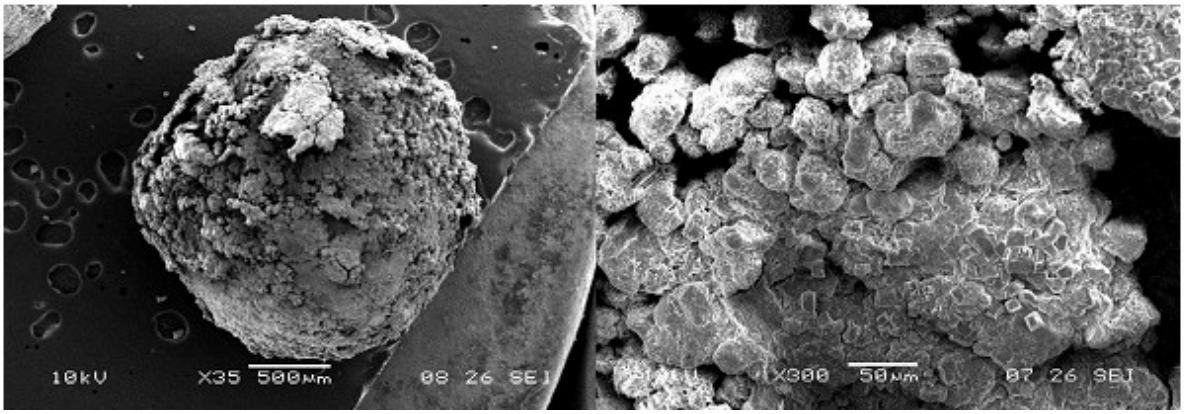

Fig. 1: Scanning electron photomicrographs of the formulation F9: a) × 35 b) × 500.

Factorial equation and response surface plot:

A 3^2 full factorial design was constructed using design expert (Version 9.2; Stat-Ease Inc., Minneapolis, Minnesota, USA) for mathematical modeling and analysis of responses where the amounts of Polymer(X_1) and crosslinking (X_2) were selected as the independent factors. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design

The polynomial equation generated are as follow:

$$\% \text{ mucoadhesion} = - 5.70370 + 0.1674X1 – 2.0000X2 – 3.8072X1X2 – 1.48148X1^2 0.2666\ X2^2 \quad \text{eq. (1)}$$

$$\% \text{ Drug content} = - 26.7740\ 7 + 0.59637X1 – 5.2666\ X2 – 2.9333\ X1X2 –6.7851\ X1^2 + 0.43733\ X2^2 \quad \text{eq. (2)}$$

$$\% \text{ Drug release} = 125.334 – 0.2334X1 + 0.6966\ X2 – 5.2666\ X2 + 0.02020X1X2 – 1.6518X1^2 – 0.6667\ X2^2 \quad \text{eq. (3)}$$

All the polynomial equations were found to be statistically significant determined using as per provision of design expert software.Equation can draw conclusion after considering magnitude of coeficient and mathematical sign carried.

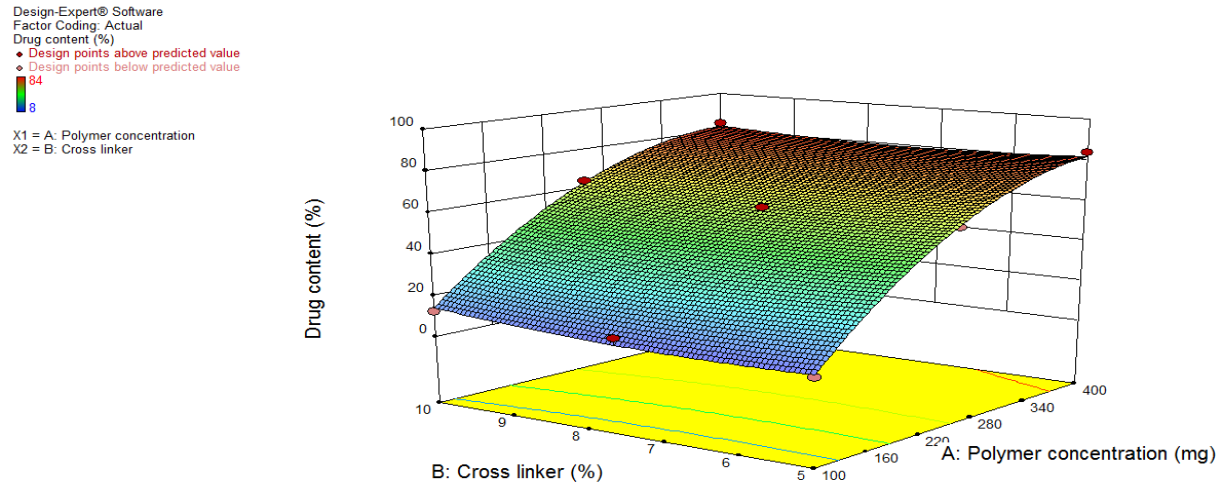

Fig. 2: Drug content 3D graph.

Results of the equation indicated that as the concentration of the X1 increases drug content increased and as the conc. of crosslinking agent decreases drug content increases.

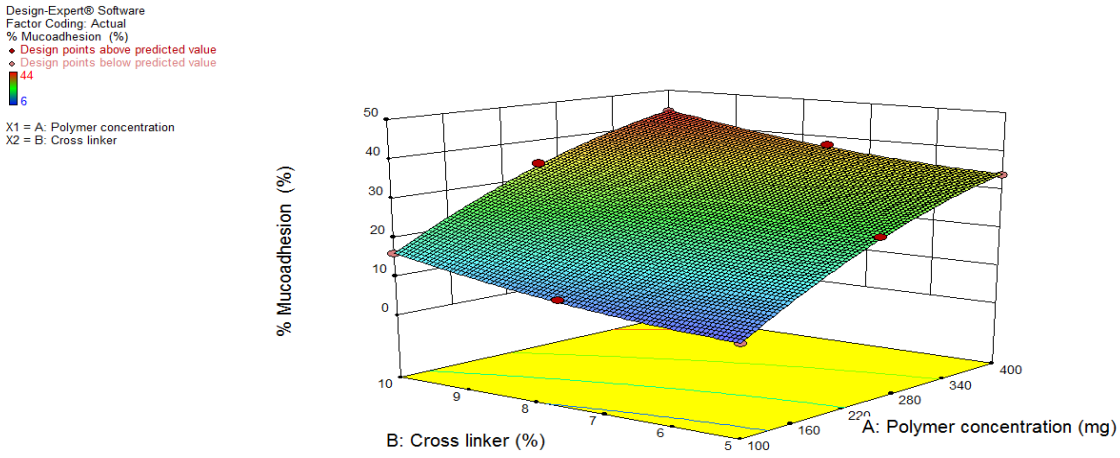

Fig. 3: Percent mucoadhesion 3D graph.

Concerning mucoadhesion, the results of multiple linear regression analysis showed that the coefficients bear a positive sign. So, increasing the amount of the polymer in the formulations increased the mucoadhesion.

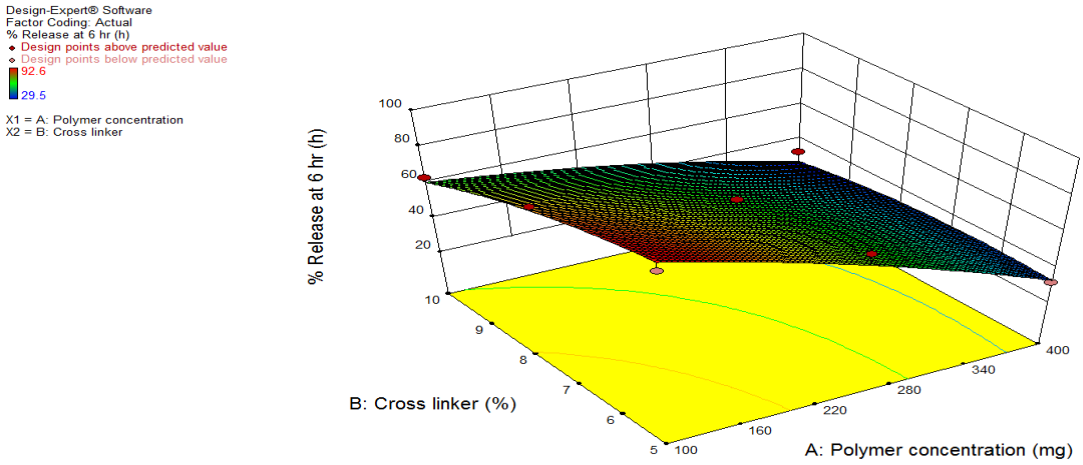

Fig. 4: Percent drug release 3D graph.

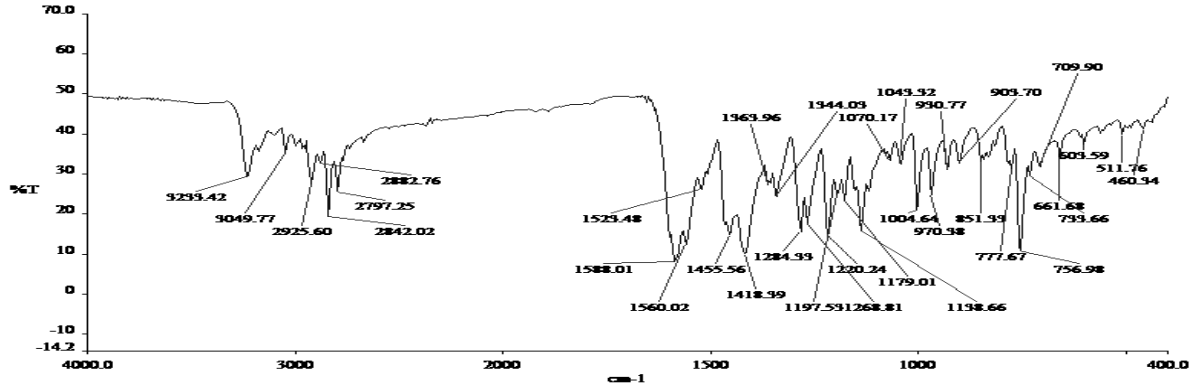

Fig. 5:FTIR of Olanzapine drug.

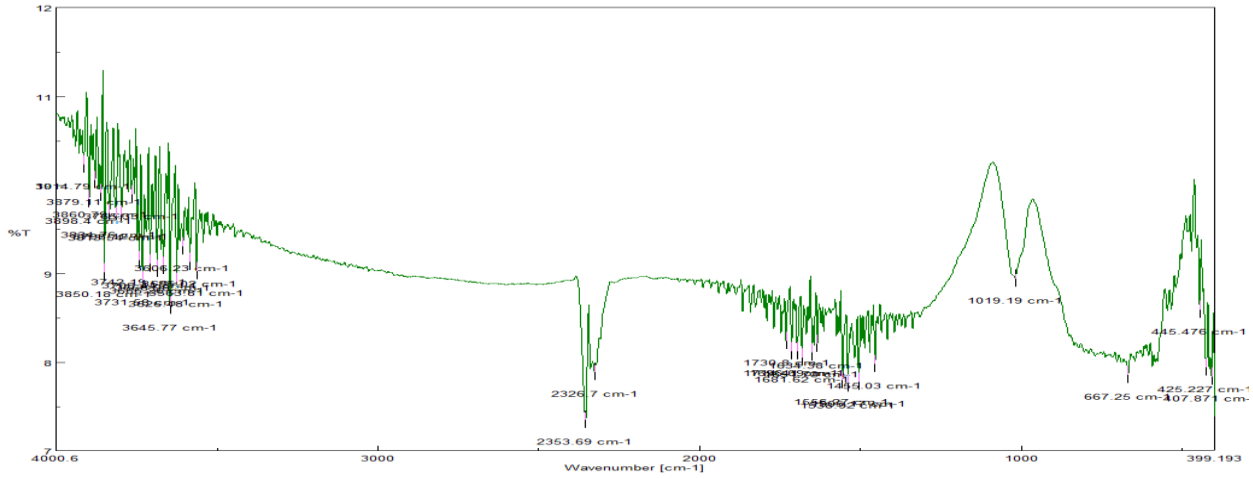

Fig. 6: FTIR of Olanzapine microsphere.

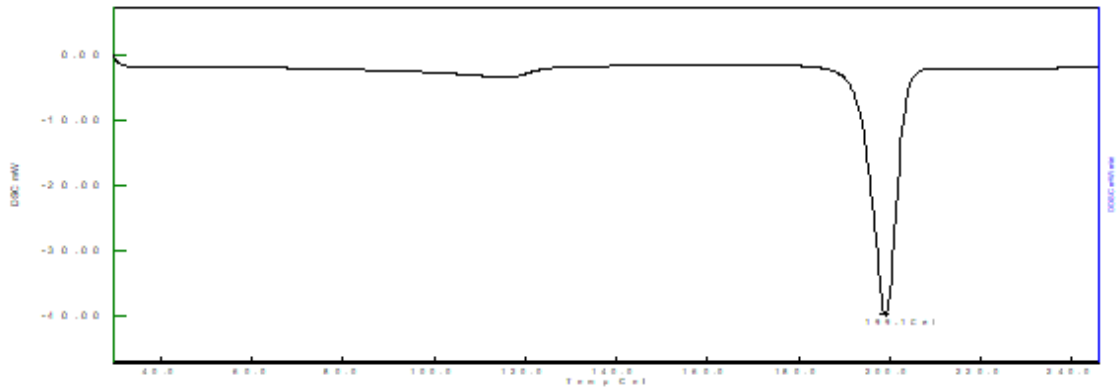

Fig.7: DSC of pure Olanzepine drug.

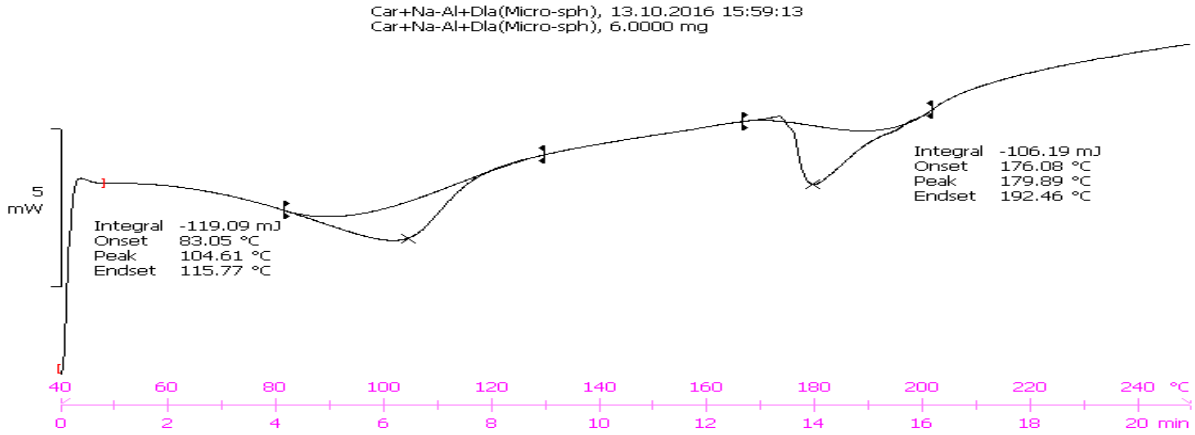

Fig.8: DSC of Olanzepine microsphere.

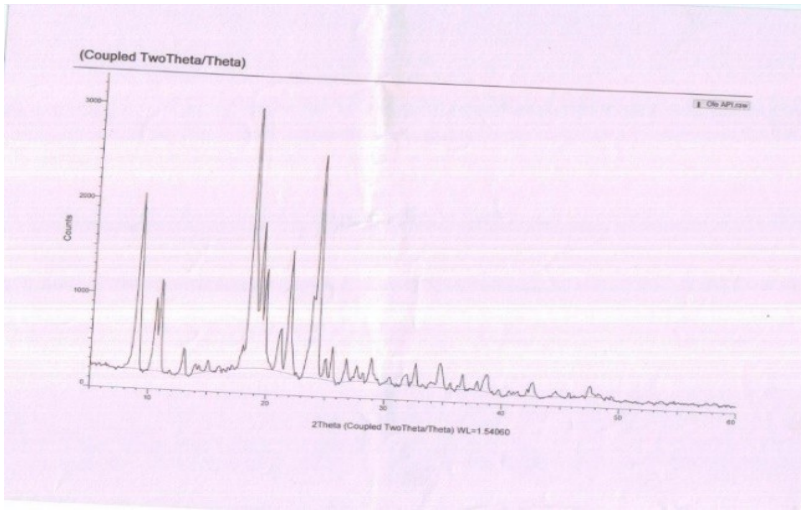

Fig. 9: XRD of Olanzapine drug.

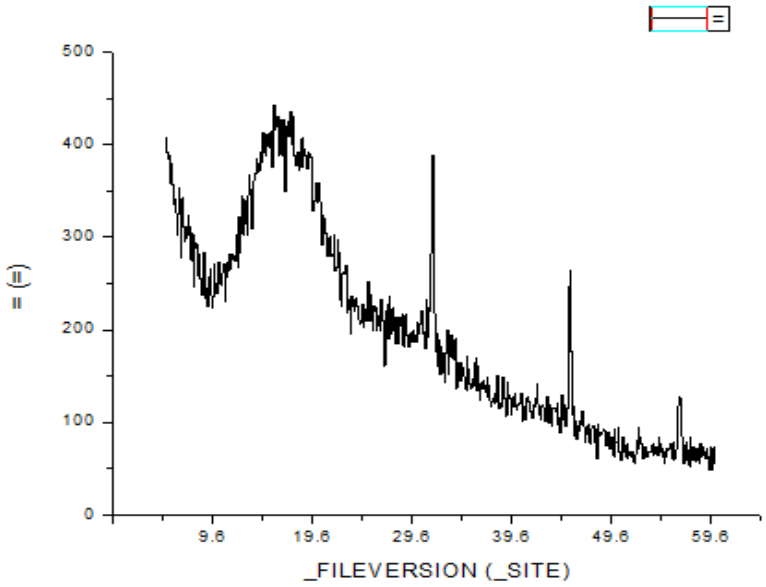

Fig. 10: XRD of Olanzapine microsphere.

Table 7: Stability studies of microsphere.

Sr. no	Duration	Drug Content (%)	No. of microsphere adhered to mucous out of 50(%)	*Invitro*dissolution (%)
1	0	82.00	43.58	30
2	1 month	82.34	43.59	30.2
3	2 month	82.45	43.66	30.21
4	3 month	82.89	43.76	30.22
5	4 month	83.11	43.88	30.23
6	5 month	83.13	43.89	30.24
7	6 month	83.13	43.89	30.24

4. CONCLUSION

The present study has been attempted to formulate a mucoadhesive microsphere of Olanzepine for oral administration for enhancing bioavailability of the drug .Olanzepine has low dissolution and low bioavailability. Hence mucoadhesive microspheres were developed to enhances the bioavailability. Microsphere formulation of OLE was prepared by using ionic gelation method using sodium alginate polymer and ca. chloride as crosslinking agent.

From the results, it can be concluded that, the IR & DSC spectra revealed that there was no interaction between sodium alginate polymer and Olanzapine, hence they are compatible. The prepared mucoadhesive microspheres of Olanzepine showed good micromeritic results. The particle size analysis revealed that all formulations having particles in the range of 657.1 µm-659.1 µm**.** Among all the formulation F9 was selected as best formulation which showed the

good entrapment efficiency (86.1 %), good mucoadhesion in 8 hr. (88%) and good drug release profile in 6h (92.23%). In vitro drug release data followed Higuchi model with regression values ranging from 0.9009 to 0.9873. SEM analysis of the F9 microspheres revealed that the formulation was spherical and rough surface morphology. The prepared mucoadhesive microspheres of Olanzepine showed sustained release action with increased therapeutic efficacy and patient compliance.

5. ACKNOWLEDGEMENT

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