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Research Article

FORMULATION AND CHARACTERIZATION OF OLANZEPINE-LOADED MUCOADHESIVE MICROSPHERES

MADHURI T DESHMUKH[1]*, SHRINIVAS K MOHITE[2]

[1]Department of Pharmaceutics, [2]Department of Pharmaceutical Chemistry, Rajarambapu, College of Pharmacy, Kasegaon, Maharashtra, India. Email: madhurideshmukh9@yahoo.com

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ABSTRACT

Objective: The objective of this research was to formulate and evaluate olanzapine (OLE) mucoadhesive microsphere prepared using carbopol and sodium combination. OLE having extensive hepatic first pass metabolism and low bioavailability problem, determined the need for the development of sustained release formulation.

Methods: OLE mucoadhesive microspheres were prepared by ionic gelation method. OLE mucoadhesive microspheres were prepared by ionic gelation method by using calcium chloride as crosslinking agent. The OLE mucoadhesive microsphere was characterized by particle size measurement, process yield, morphology of microsphere, drug entrapment efficiency, mucoadhesion test, differential scanning calorimetry, powder X-ray diffraction, Fourier transforms infrared (FTIR) study and *in-vitro* drug release.

Results: The OLE mucoadhesive microsphere having mean particle size ranged from 546.0 µm to 554.3 µm, and the entrapment efficiencies ranged from 73% to 96%. All the olanzapine (OLE) microsphere batches showed good *in-vitro* mucoadhesive property ranging from 75.89% to 96.47% and in the *in-vitro* wash off test ranging from 68.12% to 81.3%. FTIR studies indicated the no drug-polymer interactions in the ideal formulation F9. There were no compatibility issues, and the crystallinity of OLE was found to be reduced shoeing less intense peak in prepared mucoadhesive microspheres, which were confirmed by differential scanning calorimeter and X-ray diffraction studies. Among different formulations, the OLE microspheres of batch F9 had shown the optimum percent drug entrapment of microspheres. Release pattern of OLE from F9 microspheres batch followed Higuchi kinetic model. Stability studies were carried out for F9 formulation at 4°C/ambient, 25±2°C/60±5%, 40±2°C/75±5% relative humidity revealed that the drug entrapment, mucoadhesive behavior, and drug release were within permissible limits.

Conclusion: The results obtained in this work demonstrate the use of carbopol and sodium alginate polymer for preparation of mucoadhesive microsphere.

Keywords: Ionic gelation method, Gastroretentive delivery, Mucoadhesive microsphere, Carbopol.

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INTRODUCTION

Oral controlled drug delivery system such as mucoadhesive microsphere drug delivery systems used 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. Mucoadhesive microspheres have advantages such as 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].

OLE is an atypical antipsychotic thienobenzodiazepine derivative used in the treatment of schizophrenia and bipolar 1 disorder. OLE is poorly soluble in water belongs to BCS class II and having only 60% oral bioavailability. OLE undergoes extensive first pass metabolism. In this regard, our main focus of this research is to prepare sustain microspheres of OLE which provides slow release in GIT and also assures the presence of dosage form at the site of absorption. OLE has been shown to selectively bind to central dopamine D2 and serotonin (5-HT2c3 receptors and is effective against the negative symptoms of schizophrenia with a lower incidence of extrapyramidal symptoms. A second generation atypical antipsychotic having 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 this work was to formulate the mucoadhesive microsphere of OLE to improve residence of dosage form in GIT, reduced dosing frequency and enhance bioavailability in the treatment of schizophrenia.

MATERIALS AND METHODS

Materials

OLE was obtained from Enaltec Lab Private Ltd, Mumbai, India. Sodium alginate gift sample from Loba Chemical Mumbai, carbopol from Colorcon Asia Pvt. Ltd., Goa, and calcium chloride was purchased from S.B. Fine chemicals Ltd, Mumbai.

Preparation of microsphere

Ionic gelation method

The microspheres were prepared by ionotropic external gelation technique. The carbopol and alginate solution was prepared by initially dispersing the carbopol (2-6%) and sodium alginate (3% w/v) in deionized water employing mild heat (50°C) with by magnetic stirring. To this dispersion, OLE (200 mg) was added and sonicated for

30 minutes. The dispersion was then added dropsies from 20-gauge hypodermic needle fitted with a 10 ml syringe to solution of calcium chloride (5-10% w/v) stirred at 500 rpm. The gelled droplets were allowed to remain in calcium chloride solution for 30 minutes for complete curing, filtered, and washed repeatedly with deionized water to remove excess of $CaCl_2$ that might have deposited on surface of microspheres. The microspheres were then dried at 50°C under vacuum [3,7].

Experimental design

The formula optimization was done by 3^2 factorial design using design expert (Version 9.2; Stat-Ease Inc., Minneapolis, Minnesota, USA) for mathematical modeling and analysis of responses. The optimal level of variables was determined by 3^2 factorial design including center point. The significant factors selected were concentration of carbopol and cross-linker concentration examining 9 runs.

Variables for experimental designs

Independent variable

X_1 = concentration of polymer
X_2 = cross-linking agent

Dependent variable

Y_1 = Particle size
Y_2 = Entrapment efficiency
Y_3 = t% release

Particle size measurement

The size of the prepared microcapsules was measured by the optical microscopy method using a calibrated stage micrometer. Particle size was calculated using equation, $X_g = 10 \times ([n_i \times \log X_i]/N)$, Where, X_g is geometric mean diameter, n_i is number of particle in range, X_i is the midpoint of range and N is the total number of particles [8].

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 speed (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.

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

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

S. N	Batch No	X_1	X_2
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

Where Y is the dependent variable, b_0 is the arithmetic mean response of the 9 runs, and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time and (X_1X_2) represent interaction factor [9].

Process yield

Dried microspheres were accurately weighed, and considering the total amount of drug and polymers used for preparing the feed solution, the process yield was calculated, a using following formula [10].

$$\text{Entrapment efficiency} = \frac{\text{Estimated \% drug content}}{\text{Theorotical \% drug content}} \times 100$$

Morphology of microsphere

The external and internal morphology of the microspheres were studied using scanning electron microscopy in Pune University (Physics Department). The sample was loaded on copper sample holder and sputter coated with platinum [11].

Drug entrapment efficiency

Microspheres (50 mg) were powdered and suspended in 50 ml of 0.1 N HCl followed by 30 minutes sonication. The solution was kept undisturbed for 24 hrs; and filtered. The filtrate recovered was examined spectrophotometrically at 227 nm, and entrapment efficiency was calculated by the following formula [12].

***In-vitro* wash off test for microspheres**

The *in-vitro* wash off test was carried out to evaluate the mucoadhesive potential of the microspheres. In brief, a 1 cm by 1 cm rat mucosa was cut and tied onto glass slide by thread. Around 100 microspheres were spread on the wet mucosa, and the prepared slide was hung onto one of the grooves of the USP tablet disintegrating test apparatus filled with 0.1 N HCl giving regular up and down movements for 60 minutes. At the end of 60 minutes, numbers of microspheres still adhering to the intestinal mucosa were counted [13].

***In-vitro* dissolution**

The release rate of OLE from OLE microspheres was determined using USP Type II (paddle) dissolution test apparatus. The dissolution test was performed using 900 ml of dissolution medium of 0.1 N hydrochloric acids, at 37±0.5°C and a rotation speed of 50 rpm. In specified time intervals, an aliquot of 5 ml samples of the solution were withdrawn from the dissolution apparatus and with replacement of fresh fluid to dissolution medium. The samples were filtered through filter paper of 0.45 µm. The samples were analyzed at 246 nm for drug content using ultraviolet spectrophotometer. The OLE release experiment was carried out [14].

Release kinetic studies

The rate and the mechanism of release of OLE from the prepared mucoadhesive microspheres were analyzed by fitting the dissolution data into various kinetic models such as 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 [9].

Fourier transforms infrared spectroscopy (FTIR) studies

Infrared spectra for pure OLE and for the physical mixture of OLE and polymer was determined to check the intactness of the drug in the

Table 2: Formula and composition with process variables

Ingredient	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug (olanzapine mg)	200	200	200	200	200	200	200	200	200
Polymer (carbopol and sodium alginate) g	4	5	6	4	5	6	4	5	6
Cross-linking agent g	1	1	1	1.5	1.5	1.5	2	2	2
Distilled water	100	100	100	100	100	100	100	100	100

polymer mixture using FTIR - spectrophotometer. The samples were analyzed between wave numbers 4000 and 400/cm resolution [15].

Differential scanning calorimeter (DSC) studies

The thermal behavior of pure OLE and OLE 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 [16,17].

X-ray diffraction study

X-ray diffractogram of the OLE and OLE-loaded microspheres were recorded by a diffractogram (Brucker AXS D8) using Cu line as a source of radiation which was operated at the voltage 40 KV and the current 40 mA. All samples were measured in the 2θ angle range between 5° and 60° [11].

Stability study

Stability studies were carried out for OLE 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].

RESULT AND DISCUSSION

The OLE microsphere was 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 cross-linking agent. The dependant variables selected were entrapment efficiency, % mucoadhesion, and % drug release. Two factors affecting the experimental responses and three factors were selected as independent variables at three levels (−1, 0 ,+1) as shown in (Table 1). Polynomial equations for individual response reflect the relationship between dependent and independent factors The model was analyzed for fitting into appropriate mathematical model and evaluated statistically for analysis of variance. The response surface analysis was carried out employing the 3D response surfaces.

Percentage yield

The percentage yield of microspheres was calculated 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 96.12% among all the batches. The production yields of microspheres prepared by ionotropic gelation method were found to be between 73% and 96% as shown in Table 3.

Particle size

The average particle size of OLE microspheres ranged from 546.0 µm to 554.3 µm. The mean particle size was significantly increased with increasing mucoadhesive polymer concentration this may be attributed to high viscosity of mucoadhesive polymer concentration (Table 3).

Morphology of microspheres

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

Entrapment efficiency

The maximum percentage yield was found of F9 batch and was noted to be 96.12% among all the batches. This may be attributed to increase in concentration of the sodium alginate polymer increased the entrapment efficiency of the microspheres due to the formation of more intact matrix network.

***In-vitro* wash off test for microsphere**

To assess the mucoadhesive property of OLE mucoadhesive microspheres, *in-vitro* wash off test was carried out for all batches, and the results are shown in Table 4. The study of *in-vitro* wash off test revealed that all the batches of prepared microspheres had good mucoadhesive property ranging from 68.12% to 81.3%. This may be attributed that on increasing the polymer concentration, the mucoadhesive property of the microspheres also increased (Fig. 3).

***In-vitro* drug release studies**

The *in-vitro* drug release studies were carried out for all batches of microspheres shown in Fig. 4. Drug release from these mucoadhesive microspheres were slow, controlled release, and dependent on the nature and concentration of polymers used. Among all the formulations F9 showed good dissolution profile with 75.89%. It was found that drug release rate decreased as the concentration of polymer increased and also with increased concentration of cross-linking. Hence, it is considered as the best microsphere formulation, which seems to be a good candidate for controlled release of OLE.

Release kinetic study

The *in-vitro* drug release data were fitted into various mathematical models. The model that best fits the release data were evaluated by correlation coefficient (r). The correlation coefficient (r) value was used to choose the best model to describe the drug release from the microsphere. As the regression coefficient (r2) value of the Higuchi model was found to be higher. The r value in various models is given in Table 5. All the microsphere formulations (F1-F9) followed Higuchi model with regression values ranging from 0.9439 to 0.9971.

FTIR studies

FTIR spectrum of pure drug and mucoadhesive microsphere of drug and polymers were studied (Fig. 5). It was observed that OLE showed

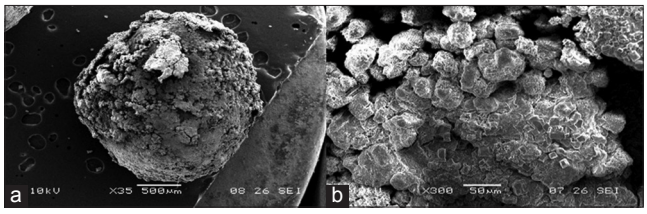

Fig. 1: Scanning electron photomicrographs of the formulation F9, (a) ×35, (b) ×500 factorial equation

Table 3: Percentage yield, particle size, percentage mucoadhesion, *in-vitro* release ((%)±S.D (n=3)) data of all batches

Formulation code	X_1	X_2	Percentage yield	Particle size	Percentage mucoadhesion	*In-vitro* release
F1	−1	−1	73.11±1.5	546±3.46	68.12±0.75	96.47±0.16
F2	−1	0	78.34±1.02	548.2±2.68	72.39±1.11	90.2±0.36
F3	−1	1	86.04±1.12	547.1±2.19	80.12±1.66	87.35±0.26
F4	0	−1	78.29±1.46	549.3±3.65	71.1±1.26	88.37±0.36
F5	0	0	83.14±2.46	550.4±1.54	79±1.46	82.58±0.41
F6	0	1	91.1±2.19	551.1±3.89	85±1.06	78.19±0.04
F7	1	−1	82.15±1.96	552.1±1.36	75.5±0.96	80.17±0.36
F8	1	0	89.1±2.01	553.2±1.27	81.3±1.46	78.64±0.29
F9	1	1	96.12±1.56	554.3±1.94	80.31±1.56	75.89±0.26

X_1: Concentration of polymer. Each sample was analyzed in triplicate (n=3) (%)±S.D (n=3), X_2: Cross-linking agent, SD: Standard deviation

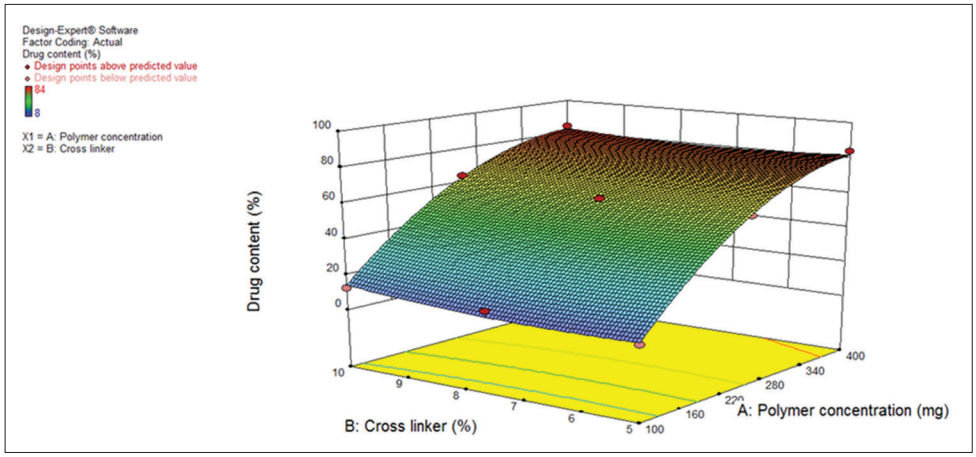

Fig. 2: Drug content 3 D graph

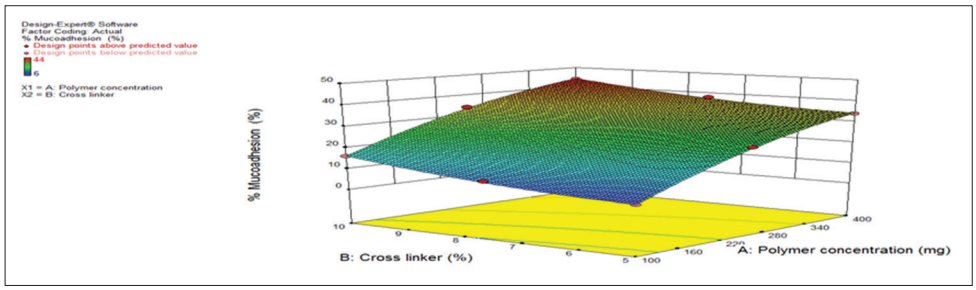

Fig. 3: Percent mucoadhesion 3 D graph

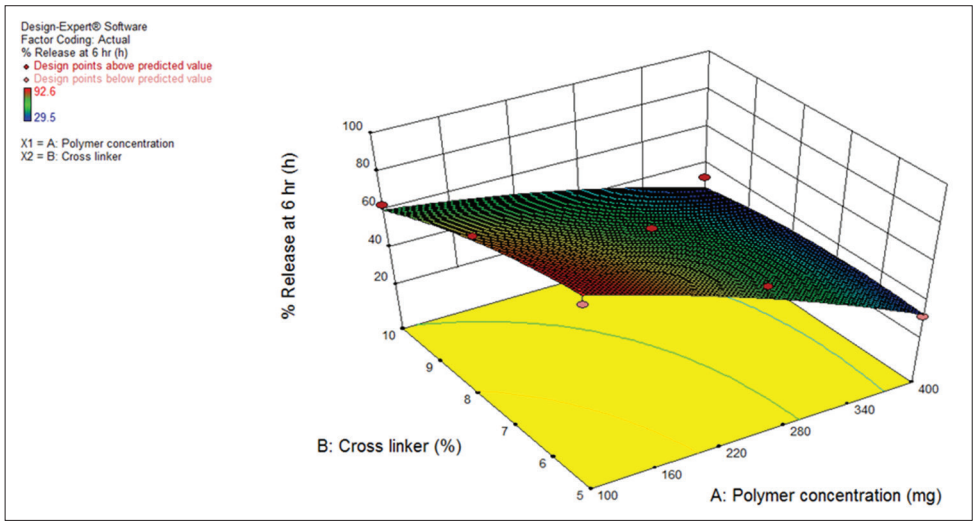

Fig. 4: Percent drug release 3 D graph

Table 4: *In-vitro* release kinetics parameters for olanzapine microspheres

Formulation code	Zero order model R^2	First-order model R^2	Higuchi model R^2
F1	0.9012	0.8932	0.9439
F2	0.9397	0.9611	0.9771
F3	0.9752	0.9673	0.9841
F4	0.9195	0.9521	0.9512
F5	0.9443	0.9613	0.9623
F6	0.9517	0.9532	0.9701
F7	0.9639	0.9194	0.9754
F8	0.9532	0.9641	0.9663
F9	0.9878	0.9853	0.9971

characteristic peak at 3337/cm for – NH group whereas sodium alginate showed – CO group at 1670/cm, – OH group at 3100/cm and – NH group at 3563/cm. While carbopol showed –CO group at 1653/cm however shift in – CO group peak of polymer (alginate) and – NH group of OLE to 1696/cm and 3563/cm suggested possibility of H-bonding between drug and polymer. DSC thermogram also showed shift in melting point of drug and no interaction between drug and polymer (Fig. 5 and 6).

DSC studies

The thermal behavior of prepared OLE microspheres was studied in comparison with thermograms of pure OLE as shown in (Fig. 7) The thermogram of pure OLE showed a sharp endothermic peak at 195°C whereas formulation containing OLE showed 2 melting endotherm

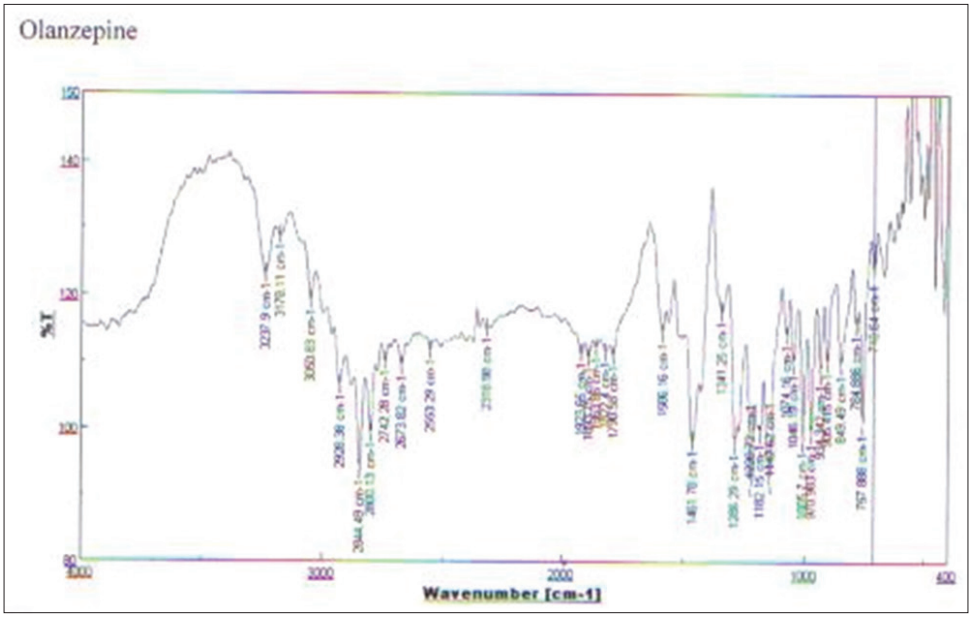

Fig. 5: Fourier transforms infrared of pure olanzapine

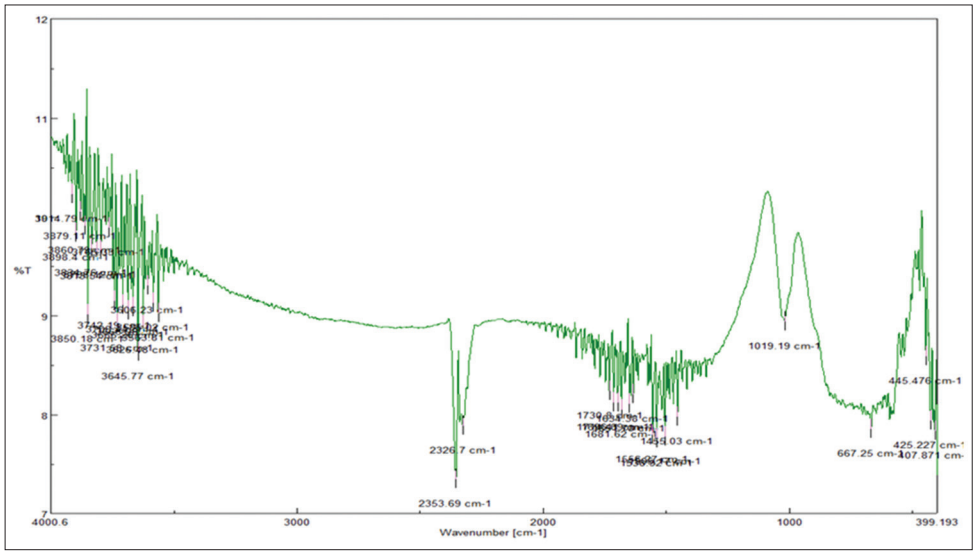

Fig. 6: Fourier transforms infrared of olanzapine microsphere

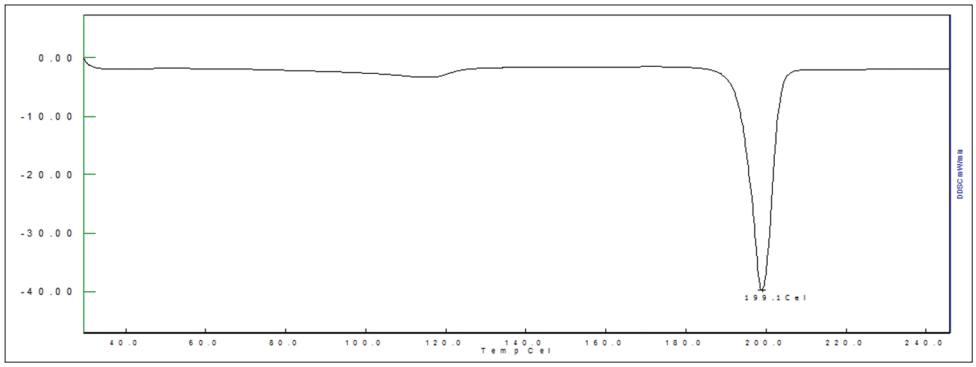

Fig. 7: Differential scanning calorimeter thermogram of olanzapine

at 126.95°C and 190.14°C which correspond to melting platinum of polymer and drug indicating that there is no interaction between drug and polymer as shown in Figs. 7 and 8.

Powder X-ray diffraction study

The X-ray diffractogram of OLE showed sharp peaks depicting a typical crystalline pattern. Physical mixtures showed less intense peaks,

Table 5: ANOVA output of the 3^2 design for optimization of microspheres

Optimized batch	Outcomes	Entrapment efficiency (%)	% mucoadhesion	Drug release
F9	R^2 value	0.9976	0.9922	0.9842

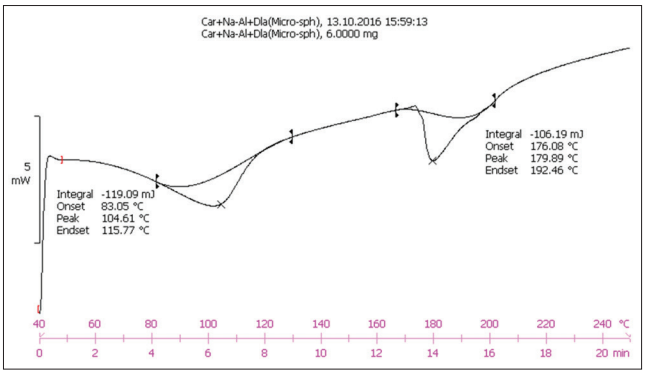

Fig. 8: Differential scanning calorimeter thermogram of olanzapine microsphere

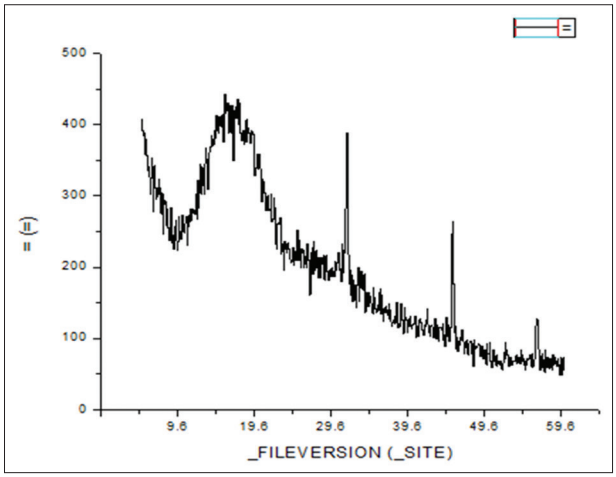

Fig. 9: Powder X-ray diffraction of olanzapine microsphere

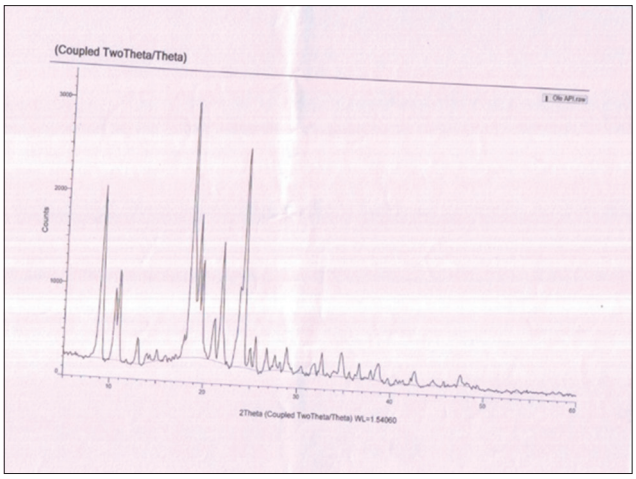

Fig. 10: Powder X-ray diffraction of olanzapine pure drug

however, OLE-loaded mucoadhesive microspheres showed less intense peaks, however, OLE-loaded mucoadhesive microspheres showed peaks, but of low intensity, revealing that some amount of OLE was changed to amorphous form. This diminished peak suggests conversion of drug into amorphous form as shown in Figs. 9 and 10.

Stability studies

Stability studies for the optimized microsphere were carried out at a temperature of 40±2°C/RH 75±5% for a period of 90 days. Formulation was 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 has good stability during their shelf life.

Factorial equation

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

Factorial equation for drug content

$$\% \text{ drug content} = 26.77407 + 0.59637\ X_1 - 5.2666\ X_2 - 2.9333\ X_1 X_2 - 6.7851\ X_1^2 + 0.43733\ X_2^2 \quad (1)$$

Where, X_1 = concentration of polymer and X_2 = cross-linking agent

Drug entrapment efficiency was found to be 61.83-70.12% (Table 3). It was found that increasing sodium alginate concentration was found to increase in content efficiency and as the concentration of cross-linking agent decreases drug content increased. Correlation coefficient was found to be 0.9976. The surface response showing effect of variables on drug entrapment efficiency is shown in Fig. 2.

Factorial equation for % mucoadhesion

$$\% \text{ mucoadhesion} = -5.70370 + 0.1674\ X_1 - 2.0000\ X_2 - 3.8072\ X_1 X_2 - 1.48148\ X_1^2\ 0.2666\ X_2^2 \quad (2)$$

Where, X_1 = concentration of polymer and X_2 = cross-linking agent

Factorial equation concerning mucoadhesion showed that the coefficients bear a positive sign. Hence, increasing the amount of the polymer in the formulations increased the mucoadhesion. The percentage mucoadhesion varied from 68.12% to 85.00% (Table 4) and showed a good correlation coefficient (0.9922). Thus, we can conclude that the amount of sodium alginate directly affects the percentage of mucoadhesion.

Factorial equation for % drug release

$$\% \text{ drug release} = 125.334 - 0.2334\ X_1 + 0.6966\ X_2 - 5.2666\ X_2 + 0.02020\ X_1 X_2 - 1.6518\ X_1^2 - 0.6667\ X_2^2 \quad (3)$$

Where X_1 = concentration of polymer and X_2 = cross-linking agent

Factorial equation concerning drug release showed coefficient of variable X_1 was found to be negative (−3.93) which indicate that release of drug from microsphere was inversely proportional to amount of polymer(sodium alginate and carbopol) and cross-linking agent, i.e., calcium chloride. The % drug release is observed to increase with decreased concentration of carbopol. The percentage drug release varied from 75.89% to 96.47% (Table 4) and showed a good correlation coefficient (0.9842).

CONCLUSION

This study has been attempted to formulate a mucoadhesive microsphere of OLE for oral administration for increasing bioavailability of the drug. The results of 3^2 full factorial design revealed that the concentration of sodium alginate significantly affected the dependent variables percentage mucoadhesion, drug entrapment efficiency, and drug release property. Microsphere formulation of OLE was prepared using ionic gelation method using sodium alginate as polymer and calcium chloride as a cross-linking agent.

From the results, it can be concluded that the IR and DSC spectra revealed that there was no interaction between polymer and drug OLE. The particle size analysis revealed that all formulations having particles in the range of 546.0-553.3 µm. Among all the formulation F9 was selected as best formulation which showed the good entrapment efficiency (96.12%), good mucoadhesion in 8 hrs (80.31%) and good drug release profile (75.89%). *In-vitro* drug release data followed Higuchi model with regression values ranging from 0.9439 to 0.9971. SEM analysis of the F9 microspheres revealed that the formulation was spherical and rough surface morphology. The prepared mucoadhesive microspheres of OLE showed sustained release action with increased therapeutic efficacy and increased patient compliance.

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