

Formulation, optimization and characterization of gastro retentive olanzepine microsphere using 32 factorial design.

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Abstract

Olanzepine microsphere prepared by external Inotropic gelation technique by using sodium alginate and carbopol 974 P as a polymer and calcium chloride as a cross linking agent. In this formulation combination of sodium alginate and carbopol 974 P used as a polymer that helps to retard the releasing the drug and increases bioavailability of drug. The Olanzepine microsphere has other characterization is in the term of drug content, drug entrapment efficiency, P XRD, Differential Scanning Colorimetry, FT-IR, SEM, *in vitro* drug release and *in vivo* drug release.

The *in vitro* release pattern of Olanzepine microsphere studied in 900 ml of 0.1 N Hydrochloric acids, using USP dissolution apparatus I. The optimized batch F7 shows greater than 80 % of drug release that is due to using carbopol polymer that helps to retarded the drug release at targeted site over the other formulation. Optimized formulation shows better antidepressant action as compare to pure Olanzepine drug.

Keywords: Ionic Gelation Technique, Microsphere, *in vitro* drug release, *in vivo* studies.

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Introduction

Microsphere are characteristically free flowing powder it is solid spherical smooth surface particle size is less than 200 μm . Microsphere are multiparticulate drug delivery system which are prepared to obtain prolonged or controlled drug delivery to improve bioavailability, stability and to target the drug to specific site at a predetermined rate. The sustained release system used to obtain maximum therapeutic efficacy, it's most important to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing less toxicity and minimum side effects. Microsphere using as a carrier for a drug its releases drug at a targeted site and shows action [1-3]. Olanzapine is antipsychotic agent belong to thienobenzodiazepines ring. It is a BCS Class II drugs which suffer from poor water solubility and high lipophilicity resulting in a highly variable oral bioavailability. Olanzapine used in treatment of schizophrenia and acute mania in bipolar disorders approved by FDA [4-6]. Despite such wide range applications, of Olanzapine are limited owing to its poor aqueous solubility and hepatic metabolism become difficult to achieve and maintain concentration of Olanzapine within therapeutic range.. Hence formulation of the mucoadhesive microsphere of Olanzapine by using Sodium alginate and carbopol 974 P polymer used to improve residence of dosage form in GIT, reduced dosing frequency and enhance bioavailability in the treatment of schizophrenia [7].

Materials and Methods

Chemicals

Table 1. Formulation code.

Formulation code	Drug (mg)	Polymer (Carbopol and Sodium alginate) gm.	Crosslinking agent gm.	Distilled water
F1	200	4	1	100
F2	200	5	1	100
F3	200	6	1	100
F4	200	4	1.5	100
F5	200	5	1.5	100
F6	200	6	1.5	100
F7	200	4	2	100
F8	200	5	2	100
F9	200	6	2	100

Olanzapine is gift sample from the Neuland Lab. Sodium alginate, from Loba chemical, Calcium chloride from SD Fine Chem.Ltd., Carbapol 974P from Coorong Asia Pvt.Ltd

Olanzapine microsphere prepared by inotropic gelation Technique

All ingredients were weighed properly according to the formulas given in Table 1.

Carbapol and alginate solution were prepared by initially dispersing the Carbapol (2%-6%) and sodium alginate (3% w/v) in water by mild heating (50°C) and stirred by magnetic stirrer. To this dispersion, Olanzapine (200 mg) was added and sonicated for 30 min. Then dispersion was then added drop wise by using 20-gauge hypodermic needle 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 but using deionized water in order to remove excess of CaCl₂ that might have deposited on surface of microspheres (Table 1). Formed microspheres were dried at 50°C under vacuum [8].

Evaluation Parameter

Drug content

10 mg Olanzapine microsphere was crushed and added to 100 ml of phosphate buffer pH 6.8. After 30 minutes the solution was filtered and from 10 ml solution 1 ml solution was withdrawn diluted up to 10 ml with phosphate buffer pH 6.8 (10 µg/ml). This solution concentration for the drug content of formulations were calculated using calibrated standard curve equation $y = 0.26418x + 0.018$. The drug content was determined at λ_{max} 227 nm by UV-spectrophotometer against blank.

Drug entrapment efficiency

50 mg microspheres were powdered and suspended in 50 ml of 0.1 N Hcl followed by 30 min. sonication. The solution was kept undisturbed for 24 hours; and filtered. The filtrate recovered was examined spectrophotometrically at 227 nm.

The Drug Entrapment Efficiency was calculated using the following formula:

$$EE (\%) = ED/AD \times 100 [9].$$

FTIR study

10 mg of the sample and 400 mg of KBr were taken in a mortar and triturated. A small amount of triturated sample was taken into a pellet maker and was compressed at 10 kg/cm² using a hydraulic press. The pellets were kept onto the sample holder and scanned from 4000 cm⁻¹ to 400 cm⁻¹ in SHIMADZU FT-IR Spectrophotometer [10].

Differential scanning calorimetry

Study was carried out to check compatibility between drug and polymer in microsphere form. Samples of 5 mg was placed in aluminum pans (Al-Crucibles, 40 µl) and sealed. The heating

was carried out between The thermograms were recorded over a temperature range of 50°C to 250°C for calibration of instrument at rate of 10°C/min under nitrogen purge gas at 50 ml/minute and samples were analyzed.

Scanning electron microscopy

The SEM analysis was carried out using a scanning electron microscope (SPPU-JEOL.). Prior to examination, samples were mounted on an aluminum stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold in vacuum. The scanning electron microscope was operated at an acceleration voltage of 5 kV and resolution of 4000. To study surface morphology, the formulation subjected to scanning electron microscopy, the micrograph depicted in Figure 1.

PXRD analysis

X-ray diffractogram of the drug and drug loaded microspheres were recorded by a diffractogram (Bruker 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°C - 60°C [11- 13].

In vitro mucoadhesion studies

The mucoadhesive properties of the microspheres were evaluated by *in vitro* wash-off test. A 1-cm by 1-cm piece of rat 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 grooves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down [14].

In vitro drug release

The release rate of Olanzapine from Olanzapine microspheres was determined using USP Type I dissolution test apparatus. The dissolution test was performed using 900 ml of 0.1 N Hydrochloric acids, at 37 ± 0.5°C and a rotation speed of 50 rpm. In specified time intervals and aliquot of 5 ml samples of the solution were withdrawn from the dissolution apparatus with replacement of fresh fluid to dissolution medium. The samples were filtered through filter of 0.45 µm. The samples were analyzed at 227 nm for drug content using UV spectrophotometer [15].

In vivo study

The protocol of the present study was approved by IAEC (proposal number RDCOP/ IAEC/ Approval / 2016 - 17/ 02 dated 8.8.2016). *In vivo* antidepressant activity was carried out using healthy Wistar albino rats and mice of either sex weighing 150 gm. to 250 gm. were used. To carry out study animals were divided in three groups as control, pure drug treated and microsphere formulation treated. The animals were provided with housing in polypropylene artificial lighting to stimulate day night cycle and access to food and water ad

libitum. Following test performed to accessing antidepressant efficacy.

Forced swim test: Forced swim tests are conducted by placing rats individually in large glass cylinders (45 × 20 cm) containing water approximately 30 cm deep. The water should be at a height such that the rat cannot escape or touch the bottom of the beaker. On day one, the rat is placed in the cylinder for 15 minutes followed by a second 5 minutes test 24 hours later. Latency to float and amount of time spent struggling are measured.

Tail suspension test: This is a behavioral test useful in the screening of potential of antidepressant drugs. Mice are suspended by their tails with tape, in such a position that it cannot escape or hold on to nearby surfaces. During this test, typically six minutes in duration, the resulting escape oriented behaviors are quantified [16,17].

Stability study

Study carried out for Olanzapine formulation as per ICH guidelines. The best mucoadhesive microspheres formulation was sealed in high-density polyethylene bottles and stored at $40 \pm 2^\circ\text{C}/75 \pm 5\%$ relative humidity (RH) for 90 days. The samples were evaluated for entrapment efficiency and percentage mucoadhesion for every 1 month to 3 months. At the intervals of each sample were withdrawn and tested.

Results and Discussion

UV visible analysis

The absorbance of the sample solution of Olanzapine shows maximum absorption at wavelength 227 nm in phosphate buffer (pH 6.8) regression coefficient is 0.9863. Which follows Beer's Lambert law (Figure 1).

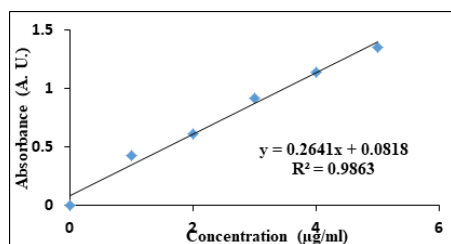


Figure 1. UV analysis of Olanzapine API.

FTIR analysis

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} . While Carbopol showed -CO group at 1653 cm^{-1} . However shift in -CO group peak of polymer (alginate) and -NH group of Olanzapine to 1696 cm^{-1} and 3563 cm^{-1} suggested possibility of H-bonding between drug and polymer (Figure 2).

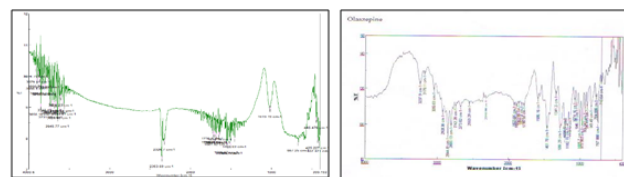


Figure 2. (A) FTIR spectra of Olanzapine microsphere (B) FTIR spectra of Olanzapine API.

Differential scanning calorimetry

The thermal behavior of prepared Olanzapine microspheres (Figure 3) was studied in comparison with thermograms of pure Olanzapine as shown. The thermogram of pure Olanzapine showed a sharp endothermic peak at 195°C whereas formulation containing Olanzapine showed 2 melting endotherm at 126.95°C and 190.14°C which correspond to melting pt. of polymer and drug indicating that there is no interaction between drug and polymer [17].

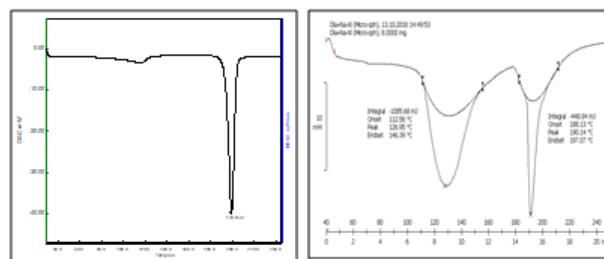


Figure 3. (A) DSC of Olanzapine API. (B) DSC of Olanzapine microsphere Scanning Electron Microscopy (SEM).

The surface of the Olanzapine microspheres was rough due to a higher concentration of polymers and uniformly dispersed of drug molecular level in matrices (Figure 4).

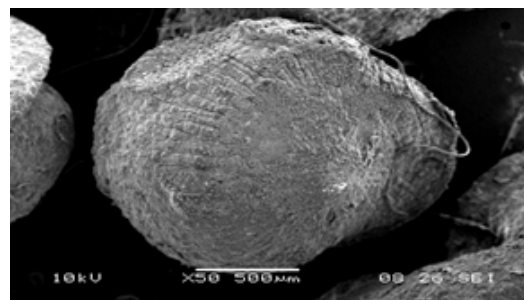


Figure 4. SEM of prepared Microsphere.

PXRD analysis

The XRD of Olanzapine showed sharp peaks depicting a typical crystalline pattern. However Olanzapine loaded mucoadhesive microspheres showed less intense peaks, revealing that some amount of Olanzapine was changed to amorphous form. Thus it can be concluded that the polymorph of the pure drug was the same as that of drug polymorph incorporated in microsphere (Figure 5).

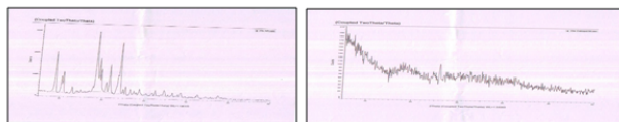


Figure 5. (A) XRD of Olanzapine API (B) XRD of Olanzapine microsphere.

Drug entrapment efficiency

From data it was observed that increase in the molecular weight of the mucoadhesive polymers increases the drug entrapment efficacy due to increase in the viscosity of the polymeric solution (Figure 6). Greater availability of binding sites in the network of polymeric chains a higher amount of drug entrapped (Table 2).

Table 2. Drug entrapment efficiency.

Formulation code	Drug content
F1	3.65 ± 0.0216
F2	3.12 ± 0.0216
F3	2.86 ± 0.0216
F4	3.9 ± 0.2160
F5	3.32 ± 0.0141
F6	3.03 ± 0.0294
F7	4.1 ± 0.2449
F8	3.56 ± 0.0432
F9	3.2 ± 0.1414

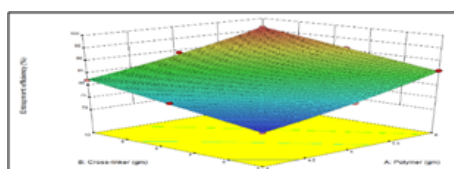


Figure 6. Drug entrapment efficiency.

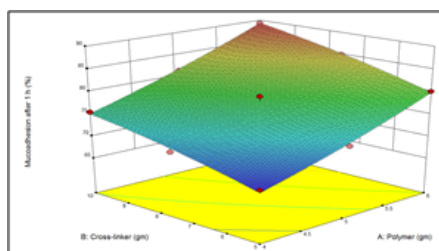


Figure 7. In vitro wash off test.

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% (Figure 7). On increasing the

polymer concentration, the mucoadhesive property of the microspheres also increased (Table 3).

Table 3. % In vitro wash off test.

Formulation code	% of microsphere adhered to mucous membrane
F1	68.12 ± 0.014
F2	72.39 ± 0.029
F3	80.12 ± 0.008
F4	71.1 ± 0.081
F5	79 ± 0.141
F6	85 ± 0.355
F7	75.5 ± 0.374
F8	81.3 ± 0.294
F9	80.31 ± 0.008

Table 4. % Drug release.

Formulation code	In vitro Dissolution (%)
F1	96.47 ± 1.0509
F2	90.2 ± 1.0816
F3	87.35 ± 1.02943
F4	88.37 ± 1.0509
F5	82.58 ± 1.04320
F6	78.19 ± 1.04546
F7	80.17 ± 1.02160
F8	78.64 ± 1.00816
F9	75.89 ± 1.43320

In vitro drug release studies

The % drug release is observed to increase with decreased concentration of Carbapol. The regression coefficient (r²) value of the was found to be 0.9971 showed excellent correlation between polymer conc. and drug release. % drug release was observed to increase with decreased concentration of Carbapol due to formation of a polymeric coat around the Olanzapine drug which retards the drug release as the concentration of Carbapol and sodium alginate polymer (Figure 8).The decrease in conc. of polymer, gives less compact beads and provides lesser no. of binding sites along alginate chains for Ca⁺² (Tables 4 and 5).

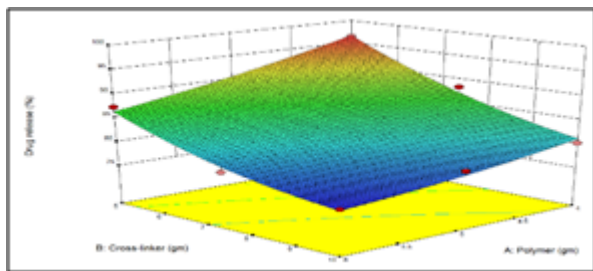


Figure 8. In vitro drug release.

Table 5. Regression coefficient (R2) value of different kinetic model.

Formulation code	Zero Model	Order	First-Order Model	Higuchi Model
	R2		R2	R2
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

In vivo study

In vivo efficiency of the prepared batch was performed in healthy normal Wistar rats by measuring the antidepressant effect produced after oral administration. The 72 mg Olanzapine microsphere equivalent to 5 mg of drug was administered and used for the study.

Table 6. Forced swimming and immobility time.

S. No.	Treatment	Swimming time (s)			Immobility Time (s)		
		15 min	30 min	60 min	15 min	30 min	60 min
1	Normal (Saline)	139.34 ± 1.57	152.67 ± 1.66	146.67 ± 2.37	59.34 ± 1.25	64.50 ± 2.62	65.67 ± 1.19
2	Std (Olanzapine;10 mg/kg, imp.)	175.834 ± 1.75	168.17 ± 1.68	166.34 ± 1.66	49.00 ± 2.06	40.00 ± 2.06	35.34 ± 2.33
3	Test (So.alginate&Carbapol)	176.50 ± 2.31	213.50 ± 2.67	227.67 ± 2.26	57.17 ± 2.61	34.84 ± 1.91	32.84 ± 2.39

Effect of acute treatments of Olanzapine API and Olanzapine - Carbapol and sodium alginate microspheres in FST:Animals from group I (normal), submitted to a 5 min

FST in second session after 24 h of first habituation session on day 1, showed increased duration of immobility and decreased swimming duration. (Table 5 and Figure 9), indicating induction of depression like state in animals. Animals from group II (Olanzapine); group III (Carbapol and sodium alginate) showed significant decrease in duration of immobility and increase in swimming duration on day 1 as compared to group I (normal). However, effectiveness of Carbapol microspheres was more than the API olanzapine (Table 6 and Figure 10). In FST., the duration of swimming of animals in treatment groups are increased as compared to the animals in normal group animal. The immobility time was significantly reduced in all treatment animals and an improved activity was seen in the animals treated with microsphere formulation.

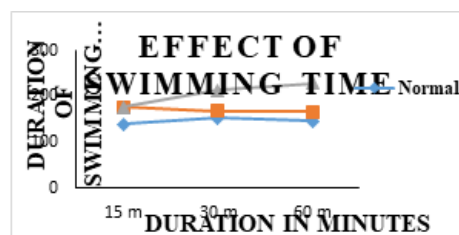


Figure 9. Effect of swimming time.

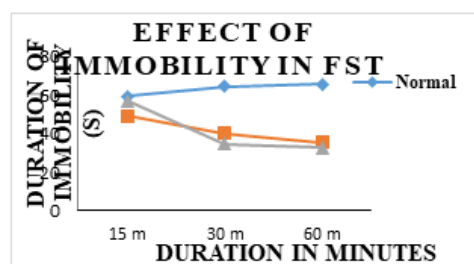


Figure 10. Effect of immobility in FST.

Table 7. Immobility in TST.

Treatment	Observations	
	Immobility Time (Before)	Immobility Time (After)
Normal(Saline)	115.84 ± 2.157	120.50 ± 2.985
Std (Olanzapine API)	118.00 ± 1.907	56.84 ± 2.552
Test(Sod.alginate&carbapol)	141.67 ± 2.270	48.34 ± 1.216

Duration of immobility of animals from group I (normal), submitted to a 6 min TST on day 1, was increased (Figure 10), indicating induction of depression like state in animals.

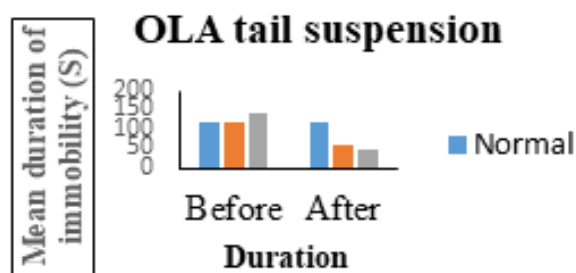


Figure 10. Effect of tail suspension test.

Conclusion

Olanzapine microsphere prepared by inotropic gelation method using carbopol and sodium alginate polymer. Carbopol and sodium alginate combination played an important role in drug release rate. Drug release rate decreased as increasing concentration of polymer. Drug Release rate it has been observed that the drug release rate decreases with time due to increasing concentration of polymer. Reason behind this issue to formation of a polymeric coat around the Olanzapine drug which retards the drug release as the concentration of Carbopol and sodium alginate polymer. The decrease in conc. of polymer, gives less compact beads and provides lesser no. of binding sites along alginate chains for Ca^{+2} . Drug Release up to 6 hour. when compared to those formulations containing concentration of pectin. The finalize batch characterize by the Drug Release test was found to be greater than 80.00 %.

Pre-formulation studies of microsphere such as determination of λ max (UV), Identification study (FTIR), thermal studies (DSC), XRD were carried out to check purity, quality of drugs used and confirm cross linking between drug and polymer. The drug-excipients compatibility study were carried out by using FTIR in order to check compatibility and found that components of the formulation were compatible with each other as there was no significant changes in IR spectra for various physical mixtures. The baseline in the DSC thermogram was observed with absence of any exothermic peak. The absence of exothermic peaks clearly indicates the stability of the mixture.

The prepared microsphere of Olanzapine was found to show good drug entrapment, percent mucoadhesion, *in vitro* release and *in vivo* release. The responses observed for all the formulations prepared were fitted to various models using Design- Expert[®] software. Advanced study such as morphology by SEM was found to show good results. Analysis of experimental results was done by using the Design Expert to optimize formulation of microsphere.

In vivo efficiency of the best found formulation of Olanzapine prepared by using Carbopol polymer was performed on healthy normal wistar albino rats by measuring the antidepressant effect produced after oral administration. When Olanzapine microsphere administered, the duration of swimming of animals in treatment groups are increased as compared to the animals in normal group and immobility time was significantly

reduced in all treatment animals and an improved activity was seen in the animals treated with combination of carbopol and sod. alginate formulation.

In mucoadhesive microspheres of Olanzapine was successfully prepared by ionic gelation method. The microspheres prepared exhibited good mucoadhesive and drug release capacity. Developed microspheres were suitable for controlled release effect after oral administration of drug. Thus the microspheres of Olanzapine show sustain the release and increase in patient compliance.

Conflict of Interest

None

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