

FORMULATION DEVELOPMENT AND EVALUATION OF ONDANSETRON MICROSPONGES FOR TOPICAL APPLICATION

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Abstract: The main aim of the study is to develop microsponges of ondansetron, microsponges are polymeric delivery systems composed of porous microspheres. These are tiny-sponge like spherical particles with a large porous surface. These may enhance stability, reduce side effects. Microsponge drug delivery system is a versatile drug delivery vehicle because of its favorable characteristics. Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and to enhance stability, reduce side effects and modify drug release. Ondansetron is a highly specific selective serotonin 5-HT₃ receptor antagonist having no activity at other serotonin receptors and low affinity for dopamine receptors. Nausea and vomiting are some of the major side effect caused by certain drug therapies like chemotherapy, radiotherapy, post-operation and radiation. Due to these symptoms, oral administration is inappropriate and intravenous administration may be unpractical. So to overcome these ondansetron microsponges for topical application were developed using various drug: polymer ratios (ondansetron : Eudragit RS100) as 2:1, 4:1, 6:1, 8:1, 10:1 and 12:1 keeping stirrer rate at 600 rpm constant. The formulations were studied for drug entrapment estimation, production yield and physical characterization. The physical characterization showed that microsponges formulated showed

reproducible results. Then the six microsphere formulations prepared as gel by using Carbopol 940, and all the gels were evaluated for their appearance, pH, drug content, rheological properties and in-vitro release. The compatibility studies were carried out using Fourier transform infra-red spectroscopy. The viscosity of all formulated ondansetron gels found in range of 3025 to 4042 cps. pH 6.1-6.4. Drug content of all prepared formulations found in the range of 92.8-93.8%, and percentage drug content showed satisfactory. Among all those F6 shows maximum drug release. The invitro drug release studies were done and best fit model was found to be zero order indicating drug release is independent upon concentration. The n value > 0.5 suggests that the drug released non-fickian release mechanism, i.e. drug released by erosion followed by diffusion controlled.

Keywords: Ondansetron, Microsphere, Gel, Eudragit RS 100, Carbopol 940, chemotherapy.

1.Introduction: Drug delivery systems (DDS) can precisely control the release rates or target drugs to a specific body site have had an enormous impact on the healthcare system. Microsphere delivery system (MDS), it is “patented, highly cross-linked, porous, polymeric delivery systems consisting of porous microspheres that can entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens, and anti-infective, anti-fungal, and anti-inflammatory agents, and then release them on to the skin over a time and in response to trigger”. Like a true sponge, each microsphere consists of a myriad of interconnecting voids within a non-collapsible structure, with a large porous surface. It is a unique technology for the controlled release of topical agents and consists of microporous beads, typically 10-25 microns in diameter loaded with active agent, depending upon the degree of smoothness or after-feel required for the end formula.

The microsphere particles themselves are too large to be absorbed into the skin and this bacterial contamination of the materials entrapped in the microsphere. As the size of the pore diameter is smaller, the bacteria ranging from 0.007 to 0.2 μm cannot penetrate into the tunnel structure of the microspheres. Delivery system comprised of a polymeric bead having network of pores with an active ingredient held within was developed to provide controlled release of the active ingredients whose final target is skin itself. MDS technology is being used in cosmetics, over-the-counter (OTC) skin care, sunscreens and prescription products. The system

was employed for the improvement of performance of topically applied drugs.

Microsponges are porous, polymeric microspheres that are used mostly for topical and recently for oral administration. Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release. The Microsponge systems are based on microscopic, polymer-based microspheres that can bind, suspend or entrap a wide variety of substances and then be incorporated into a formulated product, such as a gel, cream, liquid or powder.

The present study involves the formulation and evaluation of ondansetron microsponges formulation by using polymer Eudragit RS 100. Ondansetron is a selective serotonin 5-HT₃ receptor antagonist. Ondansetron is a competitive serotonin type 3 receptor antagonist. It is effective in the treatment of nausea and vomiting caused by cytotoxic chemotherapy drugs and has reported anxiolytic and neuroleptic properties. Microsponges are prepared by using liquid-liquid suspension polymerization method, Quasi-emulsion solvent diffusion methods.

2.Materials and Methods:

2.1.Materials: Ondansetron hydrochloride was a gift sample from Dr.Reddy's laboratories limited, Hyderabad. Poly vinyl alcohol, Eudragit RS100, Dichloromethane, Carbol940, polyethylene glycol are obtained from SD fine chemical limited, India. Di butyl phthalate is obtained from VTC product Bombay. Ethanol is obtained from Merck. All the solvents used were analytical grade.

2.2.Methods:

2.2.1.Determination of absorption maxima: For the determination of absorption maxima stock solution was prepared by dissolving 100mg of accurately weighed Ondansetron in 100ml of methanol to get 1mg/ml solution. Further 10ml of this solution was pipetted into 100ml of volumetric flask and diluted to 100ml with phosphate buffer 7.4 to get 100µg/ml solution. This stock solution was subjected for UV scanning in the range of 200-800 using Double beam UV-VIS spectrophotometer, the absorption maxima obtained at 216 with a characteristic peak.

2.2.2.Construction of calibration curve: From the above stock solution pipette out 2,4,6,8,1.0

and 2.0ml into a series of 10ml volumetric flask and was made up to 10ml with phosphate buffer pH7.4 to get 20,40,60,80,100 and 120 μ g/ml of Ondansertion respectively. The absorbance of the different diluted solutions was measured in a UV spectrophotometer at 216 nm. A calibration curve was plotted by taking concentration of the solution in μ g/ml on X-axis and absorbance on Y-axis and correlation co-efficient “ r^2 ” was calculated.

3. Drug Excipient compatibility studies:

3.1. Fourier Transform Infrared spectroscopy (FT-IR): Compatibility study was performed by preparing compatibility blends at different ratios of different excipients with the drug, based on tentative average weight. These blends were stored at accelerated condition of 40⁰C/75% RH. Control samples were stored at 40⁰C. The ratio of drug to excipient varies from 1:1 to 1:10 depending on the purpose of use, and the samples were kept in double lined poly-bags. The samples were evaluated for any change in the physical characteristics with reference to its controlled sample stored at 40⁰C for a period of 15 days.

4. Preparation of microsponges: Ondansertion microsponges were prepared by quasi emulsion solvent diffusion method. To prepare the internal phase, Ondansertion was dissolved in 10 ml of dichloromethane: ethanol (1:1) mixture to dissolve both the drug and the polymer and to this add dibutyl phthalate as a plasticizer. The external phase containing 200 ml of 1% (w/v) PVA in water. The external phase was placed in the vessel with propeller stirrer rotating at 600 rpm, to this internal phase added slowly. The system was thermally controlled at 25⁰C in a water bath. Agitations up to 30 min permit the formation of microsponges and continue stirring for 8h to get desired rigid microsponges. After 8h stop stirring filter the rigid microsponges through the filter paper (Whatmann filter paper 0.45 μ m), washed with distilled water and dried at room temperature. Ondansertion microsponges were prepared using various drug: polymer ratios (Eudragit RS100 used as polymer) i.e. 2:1, 4:1, 6:1, 8:1, 10:1 and 12:1 keeping stirring rate of 600 rpm.

Table 1 : Different formulae of microsphere formulations

Ingredients (mg)	F1	F2	F3	F4	F5	F6
Drug: polymer ratio	2:1	4:1	6:1	8:1	10:1	12:1

Internal Phase						
Ondansertion (mg)	200	400	600	800	1000	1200
Eudragit RS 100 (mg)	100	100	100	100	100	100
DCM : Ethanol (ml)	1:1	1:1	1:1	1:1	1:1	1:1
Di butyl phthalate (ml)	0.5	0.5	0.5	0.5	0.5	0.5
External phase						
PVA(mg)	100	100	100	100	100	100
Water in(ml)	200	200	200	200	200	200

5.Characterization of microsponges:

5.1. Microsponges shape and type: Microsponges can be visualized by SEM and optical microscope. The Morphological characterization of microsponges such as shape and surface feature were projected by using optical microscope and SEM.

5.2. Production yield: The dried microsponges of each batch are weighed separately and percentage yield is calculated by using following equation

$$\text{Percentage yield} = \frac{\text{Practical weight}}{\text{Theoretical weight}} \times 100$$

5.3. Loading efficiency: 200 mg of microsponges were accurately weighed. They were powdered and extracted with 100 ml of methanol. Further it was serially diluted with pH 7.4 phosphate buffer. The resulting solution was analysed for Ondansertion drug content by measuring absorbance in a UV-spectrophotometer at 216nm using pH 7.4 phosphate buffer as blank. The studies were carried out in triplicate. Loading efficiency (%) was calculated using the formula.

$$\text{Loading efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

6.Preparation of microsp sponge ondanserton gel:

- Accurately weighed amount of Carbopol 940 was taken and dissolved in water using propeller.
- In another beaker, microsponges containing Ondanserton (free or entrapped, equivalent to 200mg) drug dissolved in ethanol and added to carbopol solution by stirring, followed by addition of PEG 400. Neutralized the carbopol solution by slowly adding triethanolamine solution with constant stirring until the gel is formed.
- The pH of the final gel formed was determined.

Table 2: Ingredients used for ondansetron gel:

S.No	Ingredients	Quantity
1	Carbopol 940	0.5%
2	Ethanol	15gms
3	PEG	15gms
4	Triethanolamine	5gms
5	Water	Qs

7.Evaluation Parameters of ondansetron gel: Formulations of microsponges containing Ondanserton were characterized for pH using pH meter, viscosity using a Brookfield digital viscometer.

7.1.pH of Formulation: 1ml quantity of each formulation was transferred to a beaker and diluted by using distilled water to make 25ml. pH of the resulting solution was determined using digital pH meter.

7.2.Viscosity Measurement: The viscosity measurements were carried out by using Brookfield programmable DV-II LV model (Brookfield Eng.Lab., Inc.USA). The gel

sample was placed in small sample adapter. Temperature was increased in the range of 20⁰ C to 40⁰C, using water circulation jacket. The temperature sensing probe was lowered in gel and temperature of gel was recorded. Viscosity at various temperatures was recorded.

7.3.Estimation of drug content: 200 mg equivalent weight of Ondansertion microsphere gel was dissolved in 100ml of methanol. The filtrates was diluted with pH 7.4 phosphate buffer and measure the absorbance at 216nm using pH 7.4 phosphate buffer as blank. The drug content was analyzed from the calibration curve. The studies were carried out in triplicate.

7.4. *In vitro* Drug release studies: The release of Ondansertion from microsphere was investigated in pH 7.4 phosphate buffer as a dissolution medium (14ml) using Franz Diffusion cell. A sample of microsphere equivalent to 200 mg of Ondansertion was taken in the donor compartment. At fixed intervals, aliquots (5 ml) was withdrawn and replaced with fresh dissolution media. The concentration of drug released at different time intervals was then determined by measuring the absorbance at 216 nm against blank. The studies were carried out in triplicate. The *in vitro* drug release data of microspheres were tabulated and calculated.

8.Stability Studies: Stability of a drug has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and enables recommended storage conditions. Overall observations from different evaluation studies such as drug-polymer interactions, evaluation of prepared formulations and drug release studies were carried out. Based on the obtained results best formulation was subjected for further stability study. The stability study was conducted as per ICH guidelines for the period of six months at various accelerated temperature and humidity conditions of 25°C/60%RH, 40°C/70%RH, 60°C/80%RH. The accelerated stability study of the best formulations was carried out as per the ICH guidelines. The selected formulation was analyzed for the drug entrapment efficiency and *in vitro* release study at different temperatures.

Results and Discussion:

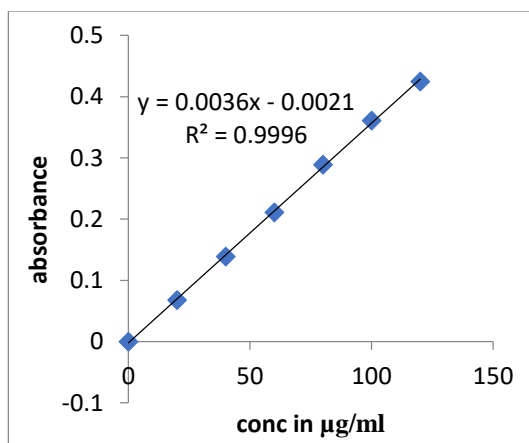
Determination of λ max:

A solution of 10 μ g/ml of Ondansetron was scanned in the range of 200 to 400nm. The drug exhibited a λ max at 216nm in Phosphate buffer pH 7.4 and had good reproducibility.

Calibration curve of Ondansetron in Phosphate buffer pH 7.4 : Table shows the calibration curve data of ondansetron in phosphate buffer pH 7.4 at 216nm. Fig shows the standard calibration curve with a regression value of 0.996, slope of 0.0036 and intercept of 0.0021. the curve was found to be linear in the concentration range of 20-120 μ g/ml.

Table 3: Calibration curve data of Ondansetron

Concentration (μ g/ml)	Absorbance
20	0.0681
40	0.139
60	0.211
80	0.289
100	0.361
120	0.425



Compatibility Studies: Drug polymer compatibility studies were carried out using Fourier Transformer Infra Red spectroscopy to establish any possible interactions of ondansetron with the polymers used in the formulation. The FT-IR spectra of the formulations were compared with the FTIR spectra of the pure drug. The results indicated that the characteristic absorption peaks due to pure Ondansetron have appeared in the formulated microsponges, without any significant change in their position after successful encapsulation, indicating no chemical interaction between Ondansetron and Polymers.

Evaluation and Characterisation of Microsponges:

Vesicle shape and Type: The surface morphology was studied by Optical Microscopy The shapes of most of the containing Ondansertan microsponges were found to be spherical from SEM analysis, as shown in figures.

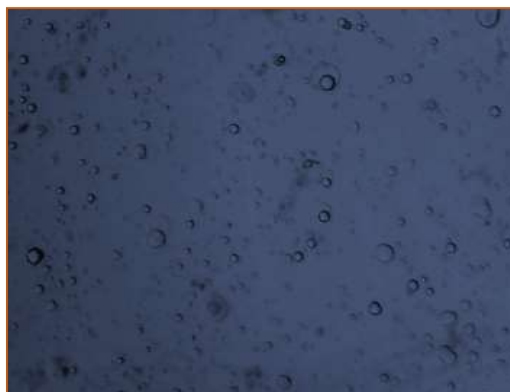


Figure 1: Photomicrograph of Ondansertan loaded microsponges at 10X

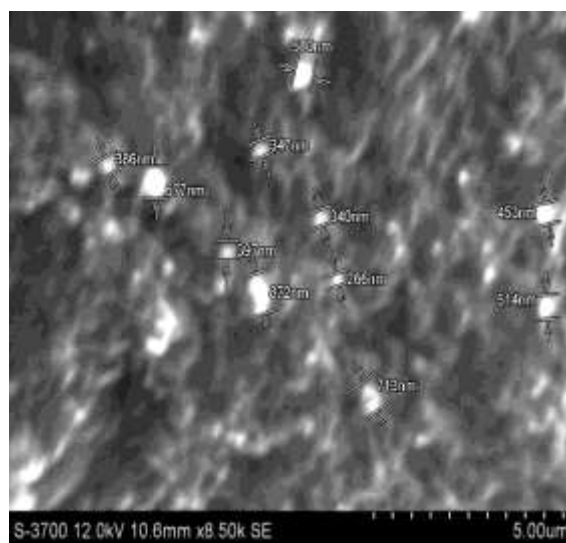


Figure 2: SEM analysis of optimized formulation

Percentage Yield: It was observed that the product yield increases with increase in drug: polymer ratio. The loss of product was due to the formation of some agglomerates and polymer adherence

to the container as a result of viscous nature of slurry. The percentage yield of the prepared microspheres is recorded in Table and displayed in Figure.

Drug Percentage Efficiency: Drug content in different formulations was estimated by UV spectrophotometric method. Basically, entrapment of the drug depends on the successful molecular association of the drug with the polymers. Percentage Drug entrapment efficiency of Ondansertone arranged from 52.35 to 78.4% for microspheres containing Eudragit as polymer. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of drug: polymer ratio. The best drug encapsulation efficiency was found for the formulation F5 and F6 with the drug polymer ratio of 10:1 and 12:1 respectively. The % drug entrapment efficiency of the prepared microspheres is displayed in Table, and displayed in Figure.

Table 4: Data showing production yield and loading efficiency of microsphere formulations:

S.No.	Formulation code	% Yield	% Drug entrapment efficiency
1	F1	76.21	52.35
2	F2	79.24	66.04
3	F3	80.42	69.24
4	F4	81.21	70.3
5	F5	87.42	72.2
6	F6	89.25	78.4

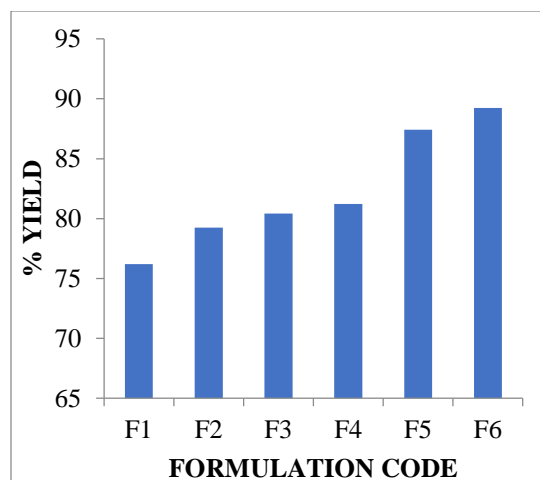


Fig3: graph for % yield vs Formulation code

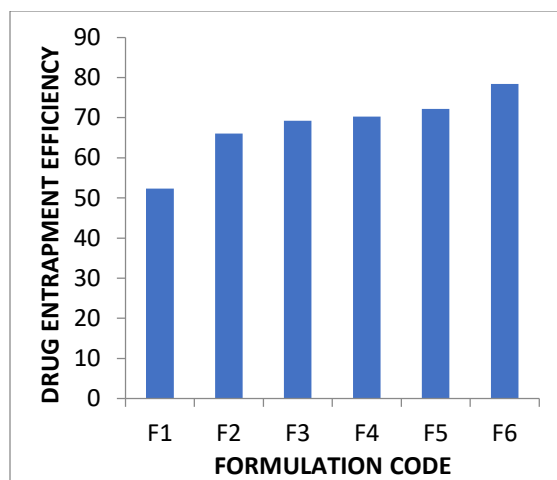


Fig 4 : graph for % drug entrapment efficiency vs Formulation code

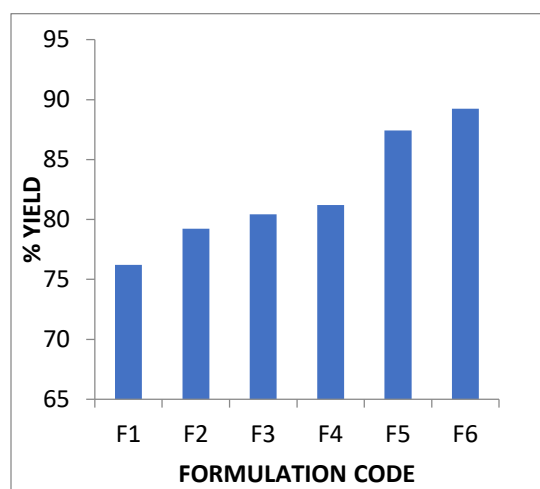


Fig 5 : graph for percentage yield vs Formulation code

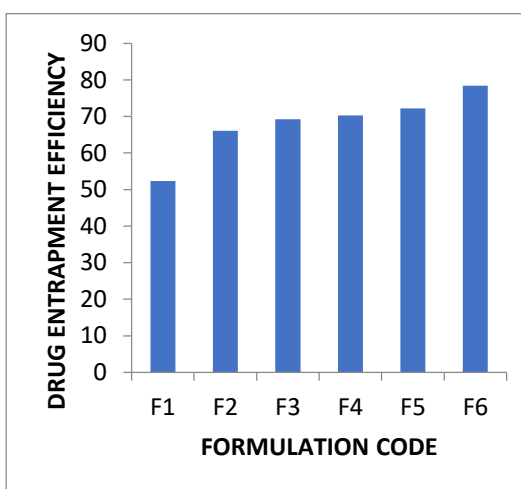


Fig 6: graph for % drug entrapment efficiency vs Formulation code

Evaluation of ondansetron gel:

pH studies: The pH of organogels was measured by using electrode based digital pH meter.

Table 5: Values of evaluation parameters of developed gel

Formulation code	pH
F1	6.1
F2	6.4
F3	6.2
F4	6.6
F5	6.6
F6	6.4

The pH value of all developed formulations of gels were in the range of 6.1– 6.4.

Viscosity measurement: The viscosity of various formulated Ondansertion gels was measured using a Brookfield viscometer. The rheological behavior of all formulated gels systems was studied. Viscosity of various formulated gels was found in range of 3025 to 4042 centipoises.

Drug content: The percentage drug content of all prepared gel formulations were found to be in the range of 92.8– 93.8 %. The percentage drug content of formulations were found satisfactory. Hence methods adopted for gels formulations were found suitable.

Table 6: Values of evaluation parameters of developed gel (viscosity and drug content)

Formulation code	Viscosity (cps)	Drug content
F1	4042	93.2
F2	4005	94.8
F3	3864	92.8
F4	3714	92.5
F5	3045	94.8
F6	3025	93.8

***In-vitro* Drug release system:** Dissolution studies of all formulations were carried out using Franz diffusion cell. The diffusion studies were conducted by using dissolution media, pH 7.4. The results of the in-vitro diffusion studies of formulations F₁ to F₆ are shown in table no. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations F₁ to F₆.

Table 7: percentage cummulative drug release for all formulations

Time(hrs)	F1	F2	F3	F4	F5	F6
1	3.8	8.2	10.4	18.4	20.25	24.2
2	6.4	10.2	14.4	27.3	31.3	38.8
3	15.5	16.5	28.3	39.7	48.6	46.1
4	24.6	25.4	42.2	45.3	50.4	52.4
5	28.2	33.4	56.5	52.4	58.2	68.4
6	39.7	47.2	68.2	78.3	79.3	74.5
7	46.4	62.2	74.7	81.9	85.6	94.2

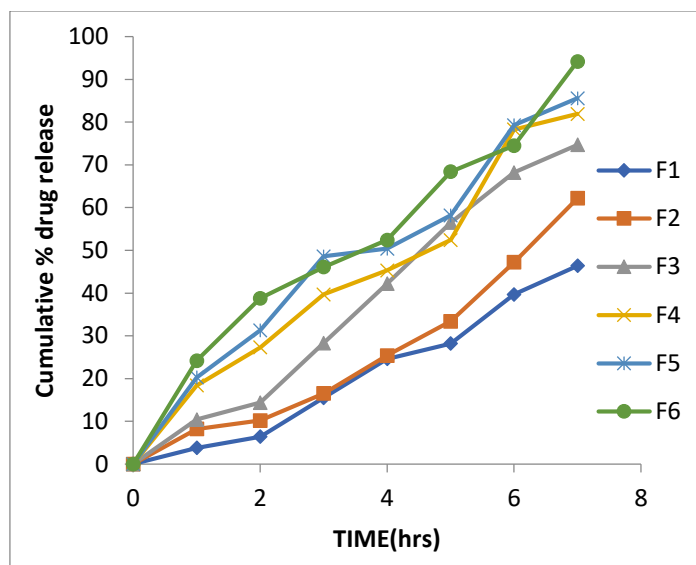


Fig7: Dissolution graph for formulation F1-F6

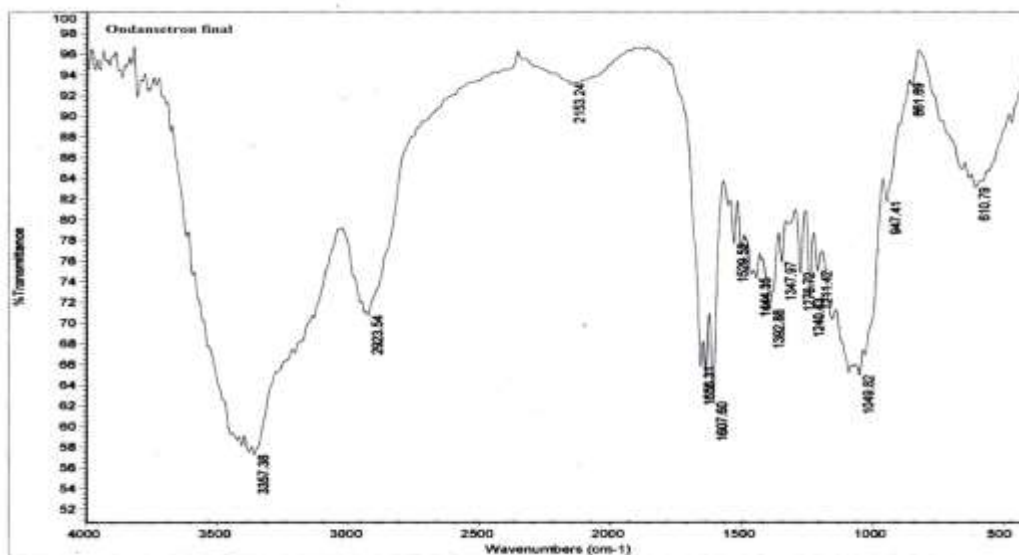


Fig8: FTIR Spectra of Ondansetron Final formulation

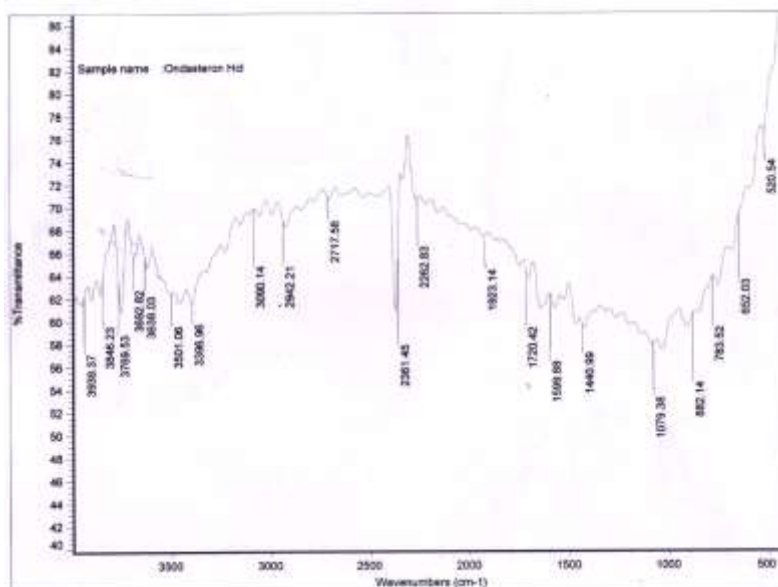


Fig 9: FTIR Spectra of Ondansetron pure drug

Summary and Conclusion: In the present study, an attempt has been made to formulate ondansetron microsponges which can be expected to improved performance for topical application to reduce irritation and to increase flexibility and for extended release of active compound. These enhances stability, reduce side effects, and modify drug release. For the formulation, biocompatible polymer Eudragit RS 100 and chosen in varying proportions with the drug. Quasi emulsion solvent diffusion method was used to prepare microsponges employing different solvent to dissolve the drug and the polymer. And then microsphere ondansetron gel was prepared using carbopol 940, and PEG 400. The prepared formulations were characterized for their production yield, loading efficiency, shape and type, drug entrapment efficiency, drug release studies. The microsphere ondansetron gel was characterized for pH, viscosity , drug content, and *invitro* drug release studies were determined.

From the result it was concluded that Microsphere containing Ondansetron was prepared by quasi emulsion diffusion method using Eudragit RS100. All the microsphere formulations were subjected for drug entrapment estimation and found results were found to be reproducible. The IR spectral analysis suggested that there was no interaction between the drug and formulation additive. Among all those formulations F6 shows maximum drug release.

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