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Disigning sustained release formulation of microsphere drug delivery systems by using tropisetron as a model drug

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ABSTRACT

Tropisetron is a 5-HT_3 (5-hydroxytrypamine$_3$) receptor antagonist used for prevention of chemotherapy-induced nausea and vomiting. The ethyl-cellulose, sodium alginate and HPMC K15M loaded microspheres were successfully prepared by using ionic gelation technique. All the formulations were evaluated for various parameters such as particle size analysis, drug content, drug entrapment efficiency, compatibility study, *in vitro* dissolution study, release kinetic study and stability study. The prepared microspheres had good spherical geometry with smooth and the particle size of a microsphere was determined by optical microscopy technique and all the batches of microspheres showed uniform size distribution. The *in vitro* dissolution studies showed that Tropisetron microspheres formulation F_{10} showed better sustained release effect over a period of 12 hours that contains 30% of all the three polymer. The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, Higuchi model, Korse meyer Peppas and finding the R^2 values it was observed that in present study *in vitro* drug release kinetic of the best formulation followed zero-order release kinetic model and drug release mechanism is anomalous diffusion coupled with erosion. Thus, from the results of the current study clearly indicate a promising potential of the tropisetron microspheres as an alternative to the conventional dosage form and can fullfil patient need by once daily medication.

Keywords: Microsphere, Tropisetron, Ethyl cellulose, Sodium alginate, HPMC K15M

INTRODUCTION

Based on recent research advancement in the controlled release dosage forms were formulated that help to improve patient convenience, enhance patient compliance because of less frequent drug administration, which attain better therapeutic efficiency of reducing as well as designed to have sustain release, sustain action, continuous action, medication which is timed release, and act slowly or delayed action. Microspheres can be best described and defined as nearly spherical particles containing measurement sizes from 1µm to 1000µm which are free-flowing particles. As microsphere morphology permits a controllable variability in drug release which helps in enhancement of bioavailability and limits the incidence or intensity of adverse reactions.[4] Due to their spherical shape and smaller size, the microsphere could be injected easily into the body. The main goal for preparing microspheres is for controlled release of the drug. This technique of microencapsulation has been used for changing the site of absorption.[1] This technique has been used for discovering

new polymeric substances developing the new chemical entities which are preferable for continuous drug release, therapeutic efficacy improvement and its safety of drug.[2] Tropisetron, an antiemetic agent, has been used for the treatment of vomiting and nausea. Anti-emetic agents are used to treat causes of vomiting and nausea. The medicament which helps to antagonize the emetics action is known as antiemetic drugs that helps to stimulate the chemoreceptor trigger zone in the medulla acts on the gastric mucosa.[3] When orally administered, the absorption is 95% whereas bioavailability is 60-80%.[5] The half-life of antiemetic action after a single dose of tropisetron is 6-8 hours; whereas tropisetron about 8% is excreted in urine as unchanged drug, 70% metabolites and 15% excreted in feces. The requirement of clinical use for a single dose is of 5-10mg to be taken once a day. Tropisetron blocks the action of serotonin receptors at the $5HT_3$ receptor which helps in suppression of chemotherapy and radiotherapy that produce nausea and vomiting. Thus a controlled release dosage form of Tropisetron is advantageous.[6]

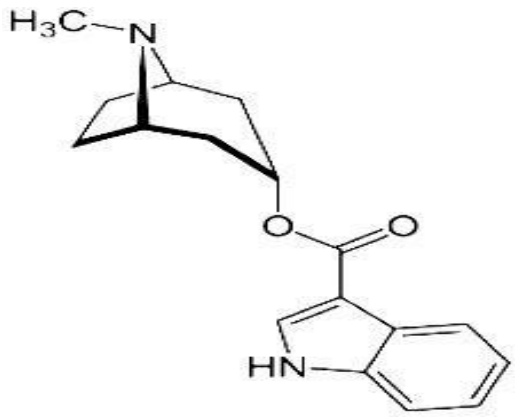

Fig 1: Structure of Tropisetron

MATERIAL AND METHOD

Tropisetron was obtained as gift sample from Dr.Reddy's laboratories Pvt. Ltd, India. Sodium alginate, HPMC K15M, Ethanol were purchased from S.D. Fine Chemicals, Mumbai, India. Ethyl cellulose was procured from Essel Fine Chemicals, Mumbai, India. Calcium Chloride was purchased from Otto Manufacturers. All chemicals were used of analytical grade.

Drug Excipient Compatibility Studies

FTIR Spectroscopy

Fourier Transform infrared spectroscopy analysis is done for the pure drug, excipient and FTIR is preformed to known interaction between drug, excipients individually and also both mixed together i.e., drug and excipient by taking a small amount of sample then it was compressed in a disc with high pressure which was then observed in peaks of sample in the range of 4000 to 500 cm^{-1} the spectrum was recorded from IR-spectral studies.[7]

Differential Scanning Calorimetry Study (DSC)

DSC is a process of thermo-analytical method in that the amount of heat is needed to increase the temperature of a sample and as a function of temperature standard is measured. Throughout the experiment, the temperature is maintained almost the same for the sample i.e. when the sample is cooled or heated it measures the energy absorbed or released. During heating or cooling the sample may undergo one or more phases.[8] The differential scanning calorimetry technique was developed in 1962 by E.S.Watson and M.J. O'Neill. The term 'DSC' was named to describe the instrument that measures energy directly and allows heat capacity measurement precisely. The Differential Scanning Calorimetry Studies were carried out for pure drug Tropisetron and optimized formulation.[9] The obtained peaks were compared which usually indicated the fusion point of the sample.

Preparation of Tropisetron microspheres by Ionic Gelation Technique

Microspheres were prepared by ionic gelation method using Tropisetron as a model drug. Tropisetron was weighed accurately and dissolved in a solution of sodium alginate using distilled water.[10, 11] Then to this HPMC K15M and ethyl cellulose added to get a viscous aqueous solution and it was stirred continuously. In a beaker 4% calcium chloride solution was prepared. Than drug and excipient dispersion was added drop wise in prepared $CaCl_2$ solution with continuously stirring using a magnetic stirrer at 50 rpm. Tropisetron microspheres were formed and further it was kept for 1 hour to curing in calcium chloride solution. After a certain time period, the prepared microsphere recovered by filtration process using Whatman filter paper and then dried at room temperature and stored in desiccators.[12, 13]

Table1: Composition of prepared Tropisetron Microspheres

Ingredients	Formulations									
	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)	F7 (mg)	F8 (mg)	F9 (mg)	F10 (mg)
Tropisetron	100	100	100	100	100	100	100	100	100	100
Sodium Alginate	400	400	100	200	350	350	-	450	450	300
Ethyl cellulose	400	100	400	350	200	350	450	--	450	300
HPMC K15M	100	400	400	350	350	200	450	450	-	300
Distilled water	Taken as required	Taken as required	Taken as required	Taken as required	Taken as required	Taken as required	Taken as required	Taken as required	Taken as required	Taken as required
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

In Vitro Drug Release Study

Accurately weigh about 150 mg of tropisetron microspheres and suspended in 900 ml of HCl buffer of pH 1.2 for about 2 hours at temperature of 37±0.5°C and at 100 rpm. The sample was withdrawn from dissolution medium at each ½ hour time interval using a 5ml syringe and analyzed using a UV spectrophotometer. Then left-over residue of microsphere from dissolution medium was filtered and suspended in 900 ml of phosphate buffer of pH 6.8 for about 10 hours. The sample was withdrawn from the dissolution medium at each 1 hour time interval using a 5ml syringe and using a UV spectrophotometer it was then analyzed.[14, 15]

Kinetics of drug release study

For analyzing the release kinetics and mechanism of drug release from the dosage form, the obtained *in vitro* release data is suited in to mathematical model i.e. Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model.[16] From the regression values of kinetic study, the order of release kinetic and mechanism of drug release obtained.[17]

Stability Studies

As per the ICH guidelines, the accelerated stability study of the best formulation was carried over a period of 90 days at 40°C ± 2°C and 75% ± 5% RH. Furthermore, this formulation which was selected had been examined at regular interval of time (every one month), for the *in vitro* release study, physicochemical parameters.[15, 16]

RESULTS AND DISCUSSION

Drug excipients Compatibility studies

Fourier transform Infrared spectroscopic analysis (FTIR studies)

To verify the compatibilities of drug and polymers used for the different formulations of Tropisetron microspheres, FTIR spectra of pure drug and physical mixture of drug and polymer used for the formulations were carried out. From these studies, it was observed that there was no shifting in the major peaks which indicated that there was no interaction took place among the tropisetron and various ingredients used in this formulation of microspheres of different formulation. Hence, the drug tropisetron and other excipients are compatible to form stable formulation under this study. The FTIR spectra of tropisetron, various polymers used and physical mixture of drug and polymer for microspheres formulation.

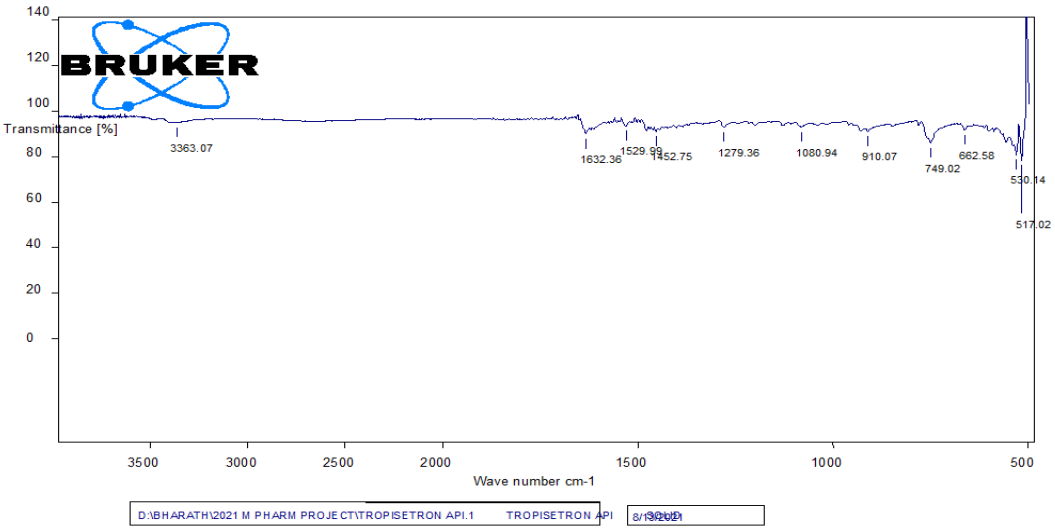

Fig 2: FTIR spectra of the pure drug Tropisetron

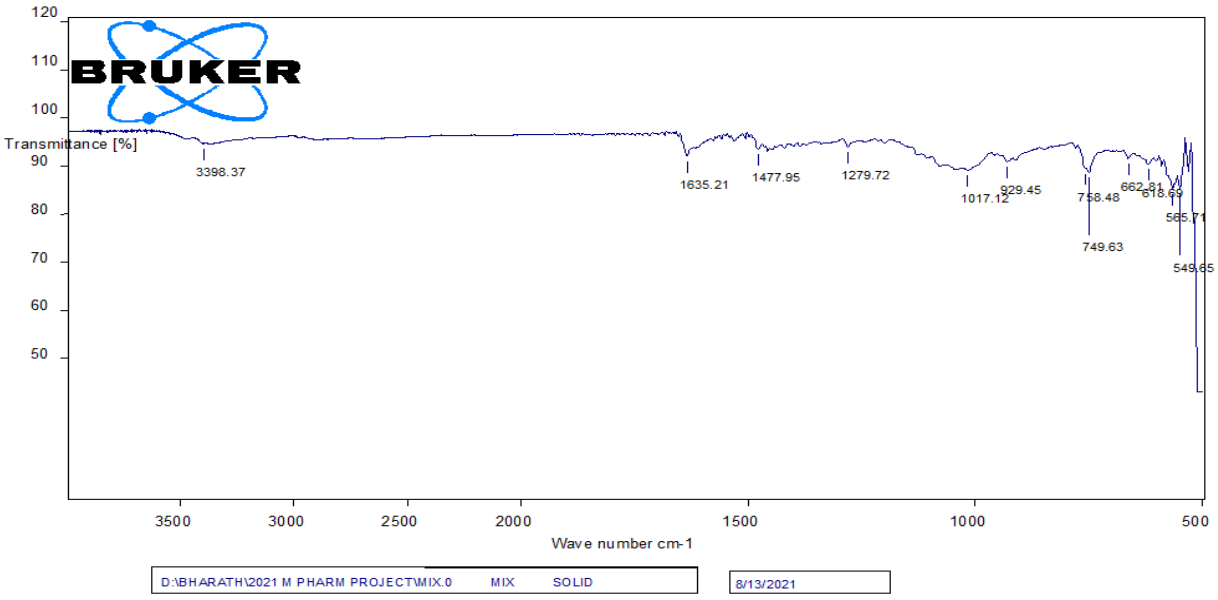

Fig 3: FTIR spectra of the Tropisetron + Sodium alginate + Ethyl Cellulose + HPMC

DSC Thermogram

From DSC Thermogram of the pure drug tropisetron and physical mixture of the polymer used for optimized formulation were observed that the endothermic peak appeared between 198.2 ℃ and 199.3 ℃ respectively which indicate that the physical mixture of optimized formulation is thermodynamically stable by the addition of tropisetron. From these DSC studies, it has been observed that this formulation is thermodynamically stable as it required marginally more heat than pure drug due to existence of various excipients with drug. It also observed that there was no shifting of peaks from endothermic to exothermic. The DSC Thermogram of Tropisetron and physical mixture of polymer used for best formulation is shown in **figure 4, 5**.

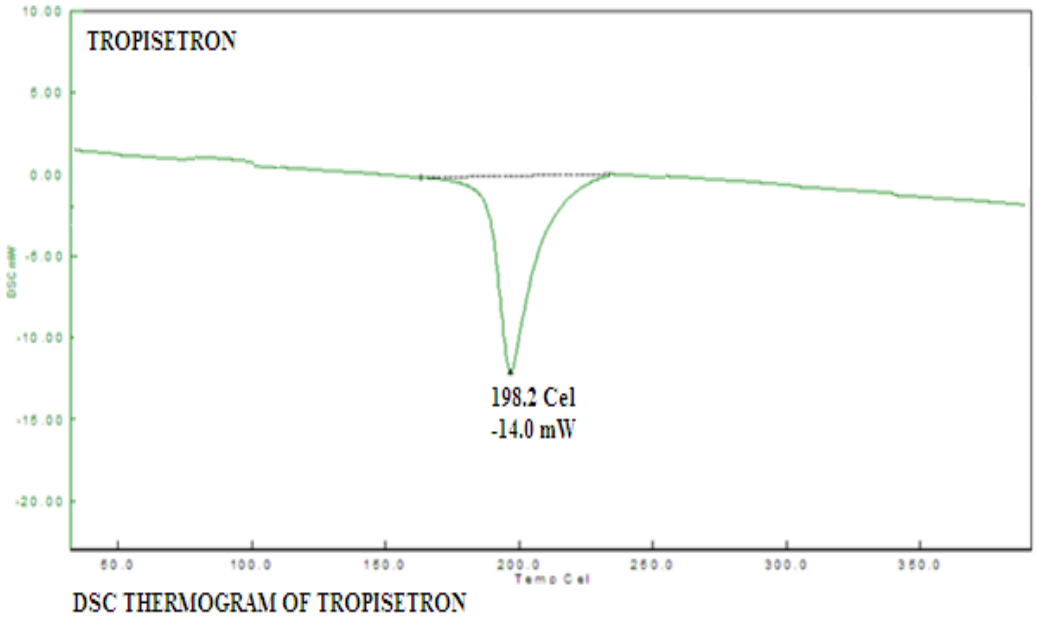

Fig 4: DSC thermogram of Tropisetron pure drug

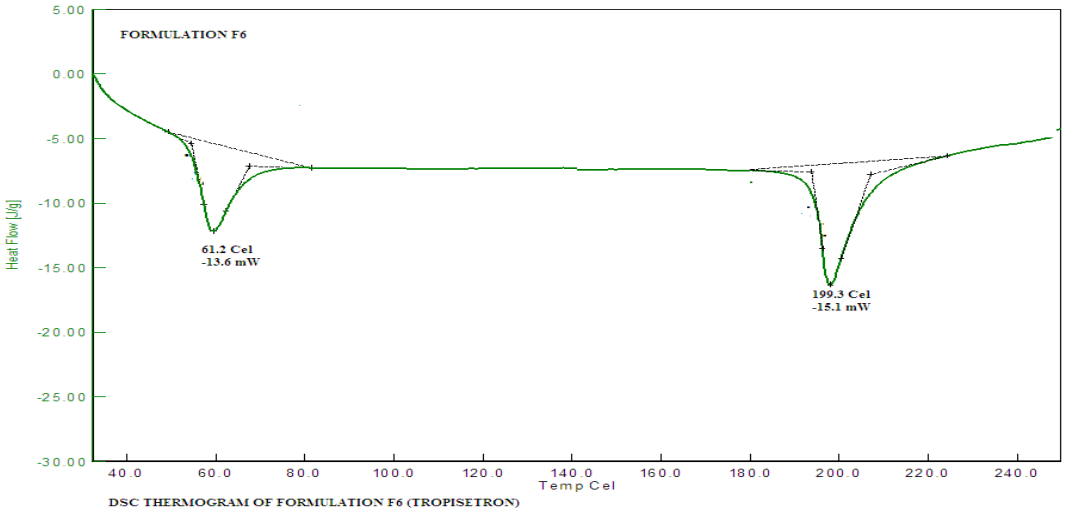

Fig 5: DSC thermogram of Tropisetron best formulation

Particle Size Analysis

Average particle size of prepared microspheres as determined by optical microscopy with the help of stage micrometer and ocular micrometer as shown in **table 2**. The mean particle size of microspheres for all formulation ranges from 281±3.23µm(F6) to 524±3.28µm(F3). The particle size showed uniformity in particle size with little deviation.

Table 2: Average Particle size of Tropisetron microspheres

Sl. No	Formulation code	Average particle size (µm)±SD
1	F1	364±2.31
2	F2	353±3.42
3	F3	524±3.28
4	F4	442±2.65
5	F5	388±2.43
6	F6	281±3.23
7	F7	445±2.21
8	F8	511±2.42
9	F9	520±1.98
10	F10	497±1.65

Percentage Yield

Percentage yield of formulation F1 to F10 was calculated and yield was found between 86.9% and 93.3% respectively. The results of all formulation F1 to F10 of prepared microspheres are shown in **table 3**. The percentage yield of all formulation was determined and results are given in table as well as represented in tabular form. All the formulation showed remarkable yield. The reduction in yield is due to loss of material during formulation of tropisetron microspheres.

Table 3: Percentage yield of Tropisetron microspheres

Sl. No	Formulation code	Theoretical yield (mg)	Practical Yield (mg)	Percentage yield (%)
1.	F1	1000	886	88.6
2.	F2	1000	905	90.5
3.	F3	1000	859	86.9
4.	F4	1000	894	89.4
5.	F5	1000	912	91.2
6.	F6	1000	858	89.8
7.	F7	1000	874	91.4

8.	F8	1000	933	93.3
9.	F9	1000	880	88.3
10.	F10	1000	883	92.3

The % drug content values are shown in table 3. As per results, F_{10} shows maximum value of drug content *i.e* 99.67% and F5 contained 91.75% of drug. Comparison of % drug content is shown in **figure 6** as histogram. The percentage drug content for all formulation were determined and presented in tabular column and histogram. The percentage drug content showed remarkable results according to specification. All the formulation showed percentage drug content more than 90%.

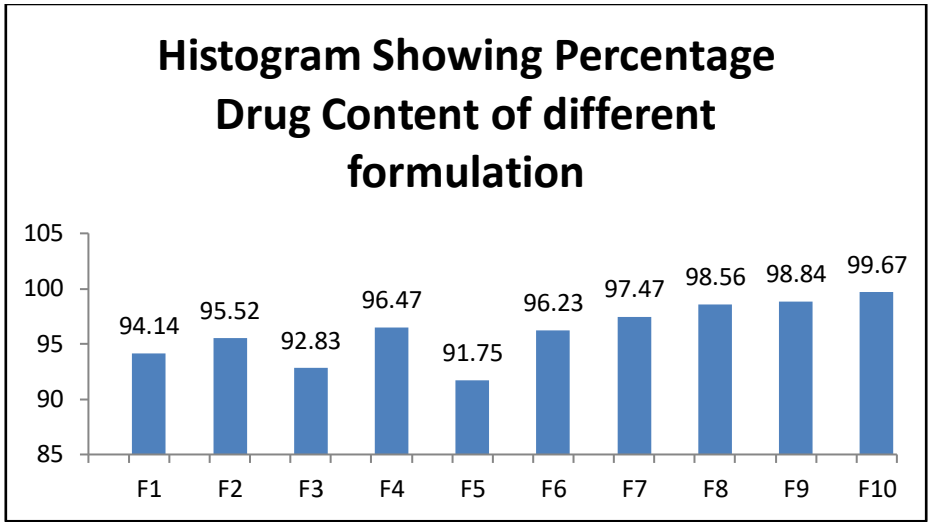

Fig 6: Comparison of % drug content of prepared Tropisetron microspheres

Determination of Drug Entrapment Efficiency

The results of % drug entrapment efficiency values has maximum value for % drug entrapment efficiency i.e. 98.71% and F_1 has shown minimum value of drug entrapment efficiency i.e. 91.78%. Comparison of % drug entrapment efficiency of microspheres were shown in **figure 7** through histogram.

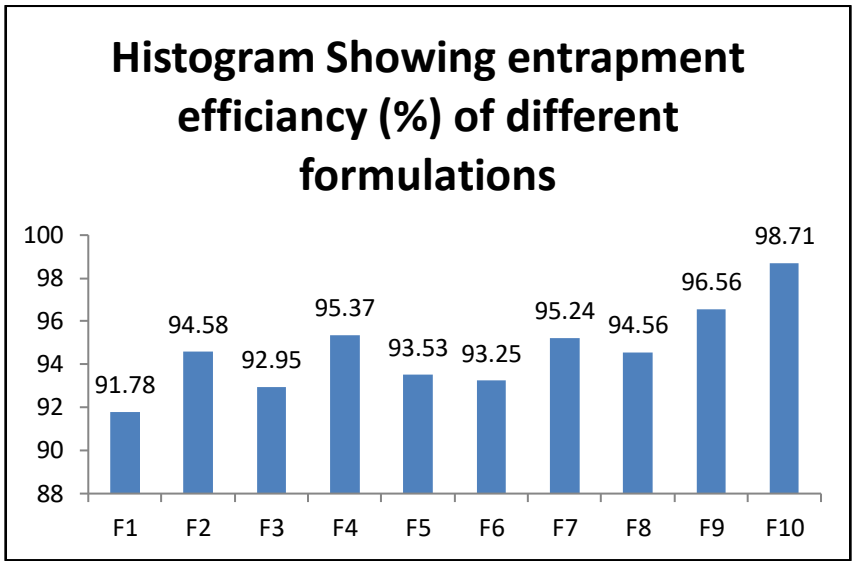

Fig.7: Comparison of % drug entrapment of prepared tropisetron microspheres

In vitro drug release study

By using a USP dissolution apparatus dissolution studies have been done on all the ten formulations of tropisetron microspheres in HCl buffer pH 1.2 for two hours and phosphate buffer of pH 6.8 was used as the dissolution medium for remaining 10 hours. It was noticed from this dissolution study that, by using higher concentration of sodium alginate, the controlled release profile of drug decreased and maximum drug released up to 8 to 9 hours whereas by increased concentration of ethyl cellulose, slow release of drug is noticed as ethyl cellulose is hydrophobic in nature. By using adequate amount of all three polymers a perfect controlled release effect is noticed up to 12 hours that is in case of F_{10} formulation which contained 30% of the entire three polymers. Comparative *in vitro* drug release data of formulation F1 to F5 are shown in **figure 8** whereas for formulation F6 to F10 the results are represented in **figure 9.**

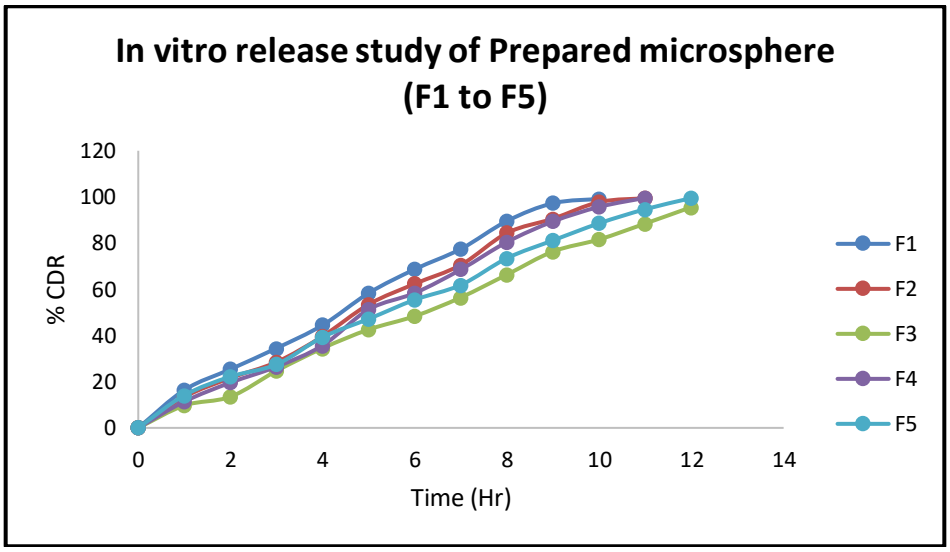

Fig 8: Comparative *in vitro* drug release of prepared tropisetron microspheres (F1 to F5)

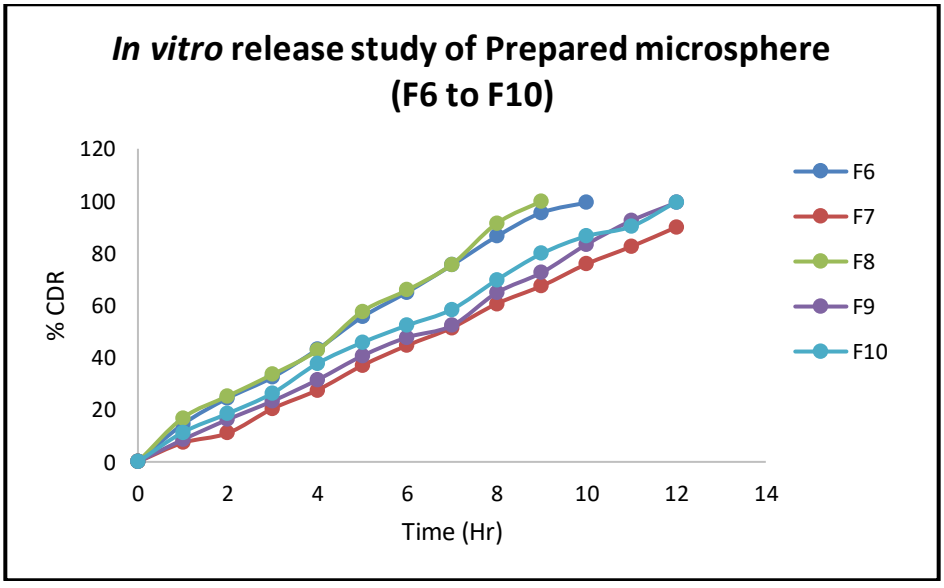

Fig 9: Comparative *in vitro* drug release of prepared microspheres of tropisetron (F6 to F10)

In Vitro Release Kinetic Studies of Tropisetron Microspheres

The calculation values for different release kinetics for all the best formulations of Tropisetron Microspheres are were calculated and regression values were represented in **table 4**.

Table 4: Regression values of *in vitro* release kinetic study best Tropisetron microspheres (F10)

Formulation	R^2value of Zero order	R^2 value of 1st order	R^2value of Higuchi model	R^2value of Hixon-Crowell model	R^2value of Peppa's model	'n' value of Peppa's model
F10	0.9987	0.6865	0.936	0.9579	0.9952	0.8964

The *in vitro* release of drug data from the best formulations **(F_{10})** of tropisetron Microspheres formulations were fitted to different kinetic models and regression coefficients were calculated. For the best formulation, the zero-order plots were found to be fairly linear as indicated by their highest regression values. The release exponent 'n' for optimized formulations were found between 0.5 to 1($0.5<n<1$), which appears to indicate a coupling of the diffusion and erosion mechanism so called anomalous diffusion. So, in present study *in vitro* drug release kinetic of the best formulation followed zero order release kinetic model and drug release mechanism is anomalous diffusion coupled with erosion.

Stability Studies

For the best formulation **F_{10}** was subjected to stability studies at 40°C /75% RH for up to 3 months. The potency of prepared microspheres was under accelerated stability conditions was within 90% to 100%. There was no change in physical appearance and was chemically stable for 3 months. The data is presented in **table 5**.

Table 5: Stability Study for F_{10} Formulation

Sl. No	Formulation	Before storage (%)	Stored at 40°C±2°C and 75%±5% RH		
			1st month (%)	2nd month (%)	3rd month (%)
1	**F_{10}**	98.12±0.36	96.74±0.45	94.67±0.52	92.13±0.48

CONCLUSION

The ethyl-cellulose, sodium alginate and HPMC K15 loaded microspheres of Tropisetron were successfully prepared by ionic gelation technique and confirmed that it is a best method for preparing Tropisetron loaded microspheres from its higher percentage yield. The formulation F_{10} has highest milligram of drug content followed by other formulations. The particle size of a microsphere was determined by optical microscopy technique and all the batches of microspheres have given uniform size distribution. The prepared microspheres had good spherical geometry with smooth as evidenced by the optical microscopy. The *In Vitro* dissolution studies showed that tropisetron microspheres formulation F10 showed better sustained effect over a period of 12 hours. It was noticed from above dissolution study that, by using higher concentration of sodium alginate, the controlled release profile of drug decreased and maximum drug released up to 8 to 9 hours whereas by increased concentration of ethyl cellulose, slow release of drug is noticed as ethyl cellulose is hydrophobic in nature. By using adequate amount of all three polymers a perfect controlled release effect is noticed up to 12 hours that is noticed in case F_{10} formulation which contained 30% of all the three polymer. The mechanism of release was determined by fitting the release data to the various kinetic equations such as zero-order, first-order, Higuchi, Korsemeyer Peppas and finding the R^2 values of the release profile corresponding to each model. It was concluded that as the polymer concentration increases, density of polymer increases that results in increased diffusion path length, in which the drug molecules have to traverse so, the drug release of F_{10} formulation takes long time than other formulations. So, in present study *in vitro* drug release kinetic of the best formulation followed zero order release kinetic model and drug release mechanism is anomalous diffusion coupled with erosion. Thus, from the results of the current study clearly indicate, a promising potential of the Tropisetron microsphere as an alternative to the conventional dosage form as it enhances bioavailability of the Tropisetron microsphere by passing the first pass metabolism and by producing sustained release effect for chemotherapy- induced nausea and vomiting. However, further clinical studies are needed to assess the utility of this system for patients suffering from nausea and vomiting.

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