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FORMULATION AND *IN VITRO* EVALUATION OF SUSTAINED RELEASE MICROSPHERE UTILIZING AN ANTIEMETIC DRUG AS A MODEL COMPOUND

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Abstract:

Tropisetron is an antagonist of the 5-HT3 (5-hydroxytryptamine3) receptor employed to avert nausea and vomiting generated by chemotherapy. The microspheres containing ethyl-cellulose, sodium alginate, and HPMC K15M were effectively synthesized by the ionic gelation method. All formulations were assessed based on many criteria including particle size analysis, drug content, drug entrapment efficiency, compatibility evaluation, in vitro dissolution testing, release kinetics analysis, and stability assessment. The synthesized microspheres exhibited excellent spherical morphology with a smooth surface, and their particle size was assessed using optical microscopy, revealing a consistent size distribution across all batches. The in vitro dissolution investigations indicated that the Tropisetron microspheres formulation TM_{10} exhibited superior sustained release characteristics for a duration of 12 hours, incorporating 30% of each of the three polymers. The release mechanism was ascertained by applying various kinetic equations, including zero-order, first-order, Higuchi model, and Korsmeyer-Peppas, and analyzing the R^2 values; the current study revealed that the optimal formulation exhibited zero-order release kinetics, with the drug release mechanism characterized by anomalous diffusion combined with erosion. The findings of the present study unequivocally demonstrate the promising potential of tropisetron microspheres as a viable alternative to traditional dosage forms, effectively meeting patient requirements with a once-daily administration.

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

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INTRODUCTION:

Recent research has led to the formulation of controlled release dosage forms that enhance patient convenience and compliance due to less frequent drug administration, thereby achieving improved therapeutic efficacy through sustained release and delayed action. Microspheres are optimally characterized as approximately spherical entities with dimensions ranging from 1µm to 1000µm, exhibiting free-flowing properties. The form of microspheres allows for a regulated variation in medication release, which aids in improving bioavailability and reduces the frequency or severity of side effects. Owing to their rounded form and diminutive dimensions, the microsphere can be effortlessly administered into the body. The primary objective of creating microspheres is to provide controlled medication release. This method of microencapsulation has been employed to alter the absorption location. This method has been employed to identify novel polymeric materials that facilitate the creation of new chemical entities, enhancing sustained drug release, therapeutic effectiveness, and drug safety.[1,2]

Tropisetron, a medication that prevents nausea and vomiting, has been utilized for therapeutic purposes. Anti-emetic medications are utilized to address the underlying causes of nausea and vomiting. The pharmaceutical agent that counteracts the effects of emesis is referred to as antiemetic medications, which stimulate the chemoreceptor trigger zone in the medulla and act on the gastrointestinal mucosa. When taken orally, the absorption rate is 95%, while the bioavailability ranges from 60% to 80%. The duration of antiemetic efficacy following a solitary administration of tropisetron is 6-8 hours; approximately 8% of tropisetron is eliminated in urine as the unmodified compound, 70% as metabolites, and 15% is expelled in feces. The clinical dosage needed is 5-10mg to be administered once daily. Tropisetron inhibits the function of serotonin receptors at the 5HT3 receptor, aiding in the alleviation of nausea and vomiting induced by chemotherapy and radiotherapy. Consequently, a regulated release formulation of Tropisetron is beneficial. [3,4]

MATERIAL AND METHOD:

Tropisetron was acquired as a complimentary sample from Dr. Reddy's Laboratories Pvt. Ltd, India. Sodium alginate, HPMC K15M, and ethanol were acquired from S.D. Fine Chemicals, located in Mumbai, India. Ethyl cellulose was obtained from Essel Fine Chemicals located in Mumbai, India. Calcium Chloride was acquired from Otto Manufacturers. All substances utilized were of analytical quality.

Drug Excipient Compatibility Studies:

Fourier Transform infrared (FTIR) Spectroscopy: FTIR spectroscopy analysis is conducted on the pure drug and excipient, as well as their individual interactions and combined effects, by compressing a small sample into a disc under high pressure, followed by observation of the resulting peaks in the 4000 to 500 cm-1 range, with the spectrum documented from IR spectral studies.[5]

Differential Scanning Calorimetry Study (DSC):

Differential Scanning Calorimetry (DSC) is a thermo-analytical technique that quantifies the heat required to elevate a sample's temperature as a function of temperature standards. During the experiment, the temperature remains nearly constant for the sample, so that when the sample is either cooled or heated, it quantifies the energy absorbed or emitted. The sample may experience one or more phases during heating or cooling. The technique of differential scanning calorimetry was established in 1962 by E.S. Watson and M.J. O'Neill. The designation 'DSC' refers to the device that quantifies energy directly and facilitates accurate measurement of heat capacity. The Differential Scanning Calorimetry analyses were conducted for the unadulterated medication Tropisetron and the refined formulation. The acquired peaks were analyzed, typically signifying the sample's melting point.[6, 7]

Preparation of Tropisetron microspheres by Ionic Gelation Technique:

Microspheres were synthesized by the ionic gelation technique with Tropisetron as a prototype medication. Tropisetron was precisely measured and dissolved in a sodium alginate solution with purified water. Subsequently, HPMC K15M and ethyl cellulose were incorporated to produce a thick aqueous solution, which was agitated incessantly. A 4% solution of calcium chloride was made in a beaker. Subsequently, the dispersion of the medication and excipient was incrementally introduced into the prepared CaCl2 solution while maintaining continuous agitation with a magnetic stirrer set at 50 rpm. Tropisetron microspheres were created and thereafter allowed to cure in a calcium chloride solution for one hour. Following a designated duration, the manufactured microsphere was retrieved by a filtration method utilizing Whatman filter paper, subsequently dried at ambient temperature, and preserved in desiccators.[8,9]

Table.1: Formulation table of prepared Microspheres of Tropisetron

Ingredients	Formulations									
	TM_1	TM_2	TM_3	TM_4	TM_5	TM_6	TM_7	TM_8	TM_9	TM_{10}
Tropisetron (mg)	100	100	100	100	100	100	100	100	100	100
Sodium Alginate (%)	40%	40%	10%	20%	35%	35%	-	45%	45%	30%
Ethyl Cellulose (%)	40%	10%	40%	35%	20%	35%	45%	--	45%	30%
HPMC K15M (%)	10%	40%	40%	35%	35%	20%	45%	45%	-	30%
Distilled water (ml)	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 (mg)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

EVALUATION

Determination of particle size:

The particle dimensions were ascertained by a calibrated optical microscopy technique utilizing a stage micrometer and an eye micrometer.

Percentage yield:

By using the following equation percentage yield can be calculated

$$\% \text{ Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Drug Entrapment Efficiency:

The microspheres were precisely weighed, holding a theoretical amount of 100mg of Tropisetron. A phosphate buffer with a pH of 6.8 was created. The microspheres were incorporated into the phosphate buffer at a pH of 6.8 and maintained for 24 hours. Subsequently, it was thoroughly agitated. Aliquots of 5 ml were extracted and appropriately diluted. The medication concentration was assessed utilizing a UV spectrophotometer. [10-11]

The drug entrapment efficiency percentage was determined utilizing the subsequent formula.

$$\% \text{ Percentage drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

***In Vitro* Drug Release Study:**

Precisely measure approximately 150 mg of tropisetron microspheres and dissolve them in 900 ml of HCl buffer at pH 1.2 for approximately 2 hours at a temperature of 37±0.5°C and at 100 rpm. The specimen was extracted from the dissolving liquid at every half-hour interval utilizing a 5ml syringe and thereafter examined with a UV spectrophotometer. Subsequently, the residual microsphere from the dissolution medium was filtered and reconstituted in 900 ml of phosphate buffer at pH 6.8 for approximately 10 hours. The sample was extracted from the dissolving media at each hourly interval utilizing a 5ml syringe, followed by analysis with a UV spectrophotometer. [12-13]

Kinetics of drug release study:

To examine the release kinetics and mechanisms of drug liberation from the dosage form, the acquired *in vitro* release data is fitted into mathematical models such as Zero order, First order, Higuchi matrix, Peppas, and Hixson Crowell models. The regression data from the kinetic investigation revealed the sequence of release kinetics and the mechanism of drug release. [14, 15]

Stability Studies:

In accordance with ICH requirements, the accelerated stability assessment of the optimal formulation was conducted over a duration of 90 days at 40°C ± 2°C and 75% ± 5% relative humidity. Moreover, the chosen formulation underwent evaluation at consistent intervals (monthly) for the in vitro release analysis and physicochemical characteristics. [16, 17]

RESULTS AND DISCUSSION:

Drug Excipients Compatibility studies

Fourier transform Infrared spectroscopic analysis (FTIR studies)

FTIR spectra were conducted to ascertain the

compatibility of the drug and polymers utilized in various formulations of Tropisetron microspheres, examining both the pure drug and the physical mixing of the drug and polymer. The experiments revealed that the primary peaks remained unchanged, suggesting no interaction occurred between tropisetron and the numerous components utilized in this microsphere formulation. Consequently, the medication tropisetron and several excipients are suitable for creating a stable formulation in this investigation. The FTIR spectra of tropisetron, different polymers utilized, and the physical combination of the drug and polymer for microsphere formulation were acquired and are illustrated in figure 1.

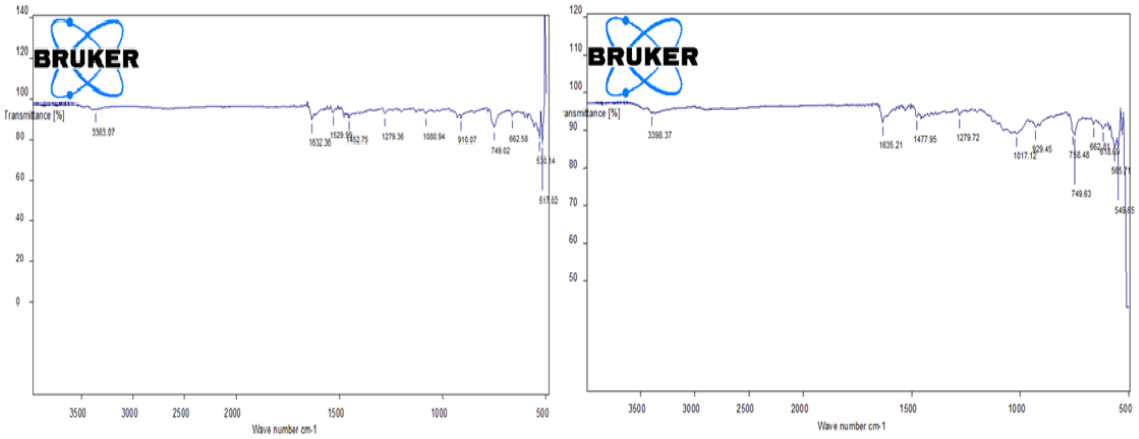

Figure 1: FTIR spectra of the Tropisetron and Tropisetron with polymers

DSC Thermogram:

The DSC thermogram of the pure drug tropisetron and the physical mixture of the polymer for the optimized formulation revealed that the endothermic peak was detected between 198.2 °C and 199.3 °C, indicating that the physical mixture of the optimized formulation exhibits thermodynamic stability due to the incorporation of tropisetron. The DSC experiments indicate that this formulation exhibits thermodynamic stability, necessitating somewhat more heat than pure medication due to the presence of diverse excipients. It was also noted that there was no transition of peaks from endothermic to exothermic. The DSC Thermogram of Tropisetron and the physical amalgamation of the polymer utilized for optimal formulation is depicted in figure 2.

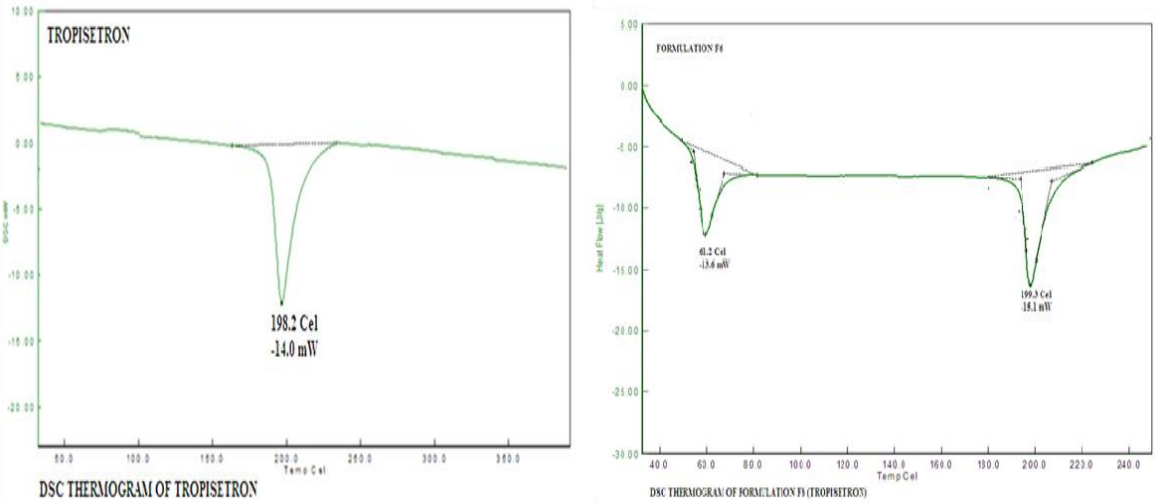

Figure 2: DSC thermogram of Tropisetron pure drug and optimized formulation

Particle Size Analysis:

The mean particle size of the synthesized microspheres was assessed via optical microscopy utilizing a stage micrometer and an ocular micrometer, as illustrated in table 2. The average particle size of microspheres across all formulations varies from 162±2.13µm (TM_6) to 413±2.18µm (TM_3). The particle dimensions exhibited consistency with minimal variation.

Table 2: Average Particle size of Tropisetron microspheres

Sl. No	Formulation code	Average particle size(µm)±SD
1	TM_1	242±1.21
2	TM_2	231±2.32
3	TM_3	413±2.18
4	TM_4	321±1.45
5	TM_5	254±1.23
6	TM_6	162±2.13
7	TM_7	323±1.31
8	TM_8	334±1.22
9	TM_9	412±2.48
10	TM_{10}	263±2.35

Percentage Yield

The percentage yield for formulations TM_1 to TM_{10} was determined, revealing yields ranging from 84.5% to 91.8%. The outcomes of all formulations TM_1 through TM_{10} of the prepared microspheres are presented in table 3 and figure 3. The yield percentage of each formulation was calculated, and the findings are presented in a table format. Every formulation demonstrated exceptional yield. The decline in yield is attributed to material loss during the formulation of tropisetron microspheres.

Table 3: Percentage yield of Tropisetron microspheres

Sl. No	Formulation code	Theoretical yield (mg)	Practical Yield (mg)	Percentage yield (%)
1.	TM_1	1000	882	88.2
2.	TM_2	1000	908	90.8
3.	TM_3	1000	854	85.4
4.	TM_4	1000	886	88.6
5.	TM_5	1000	902	90.2
6.	TM_6	1000	845	84.5
7.	TM_7	1000	905	90.5
8.	TM_8	1000	912	91.2
9.	TM_9	1000	874	87.4
10.	TM_{10}	1000	918	91.8

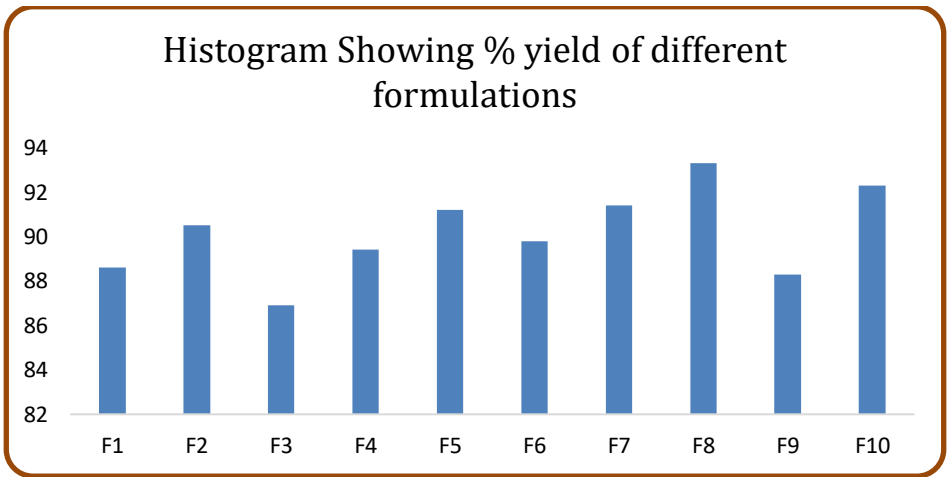

Figure 3: Comparison of percentage% yield of prepared Tropisetron microsphere

The percentage of drug content is presented in table 3. According to the findings, TM10 exhibits the highest drug content at 99.67%, while TM5 has 91.75% of the drug. The histogram in figure 10 illustrates the comparison of percentage drug content. The drug content percentages for all formulations were ascertained and displayed in a tabular format and histogram. The percentage of medication content demonstrated exceptional outcomes in accordance with the specifications. All formulations exhibited a drug content exceeding 90%.

Table 4: Drug content of tropisetron microspheres

Sl. No	Formulation Code	Drug content (%)
1.	TM_1	93.12
2.	TM_2	94.34
3.	TM_3	91.81
4.	TM_4	95.45
5.	TM_5	90.73
6.	TM_6	95.42
7.	TM_7	96.45
8.	TM_8	97.54
9.	TM_9	97.82
10.	TM_{10}	98.75

Determination of Drug Entrapment Efficiency

The outcomes of the drug entrapment efficiency percentages are presented in table 5. The table indicates that TM_{10} exhibits the highest percentage of drug entrapment efficiency at 97.62%, while TM_1 demonstrates the lowest at 90.42%. The histogram in figure 4 illustrates the comparative % drug entrapment efficiency of the microspheres.

Table 5: Drug Entrapment Efficiency of Tropisetron Microspheres

Sl. No	Formulation Code	%Drug entrapment efficiency
1.	TM_1	90.42
2.	TM_2	92.28
3.	TM_3	91.55
4.	TM_4	93.77
5.	TM_5	91.31
6.	TM_6	92.65
7.	TM_7	93.44
8.	TM_8	92.84
9.	TM_9	94.43
10.	TM_{10}	97.62

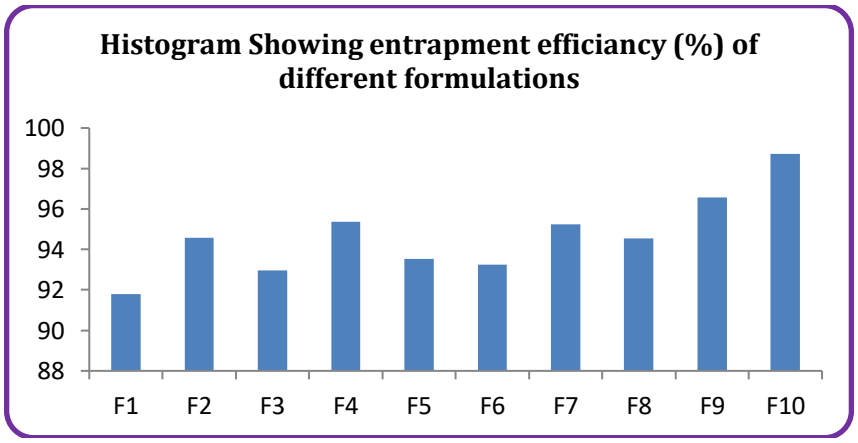

Figure 4: Comparison of % drug entrapment of prepared tropisetron microspheres

***In vitro* drug release study**

Utilizing a USP dissolution equipment, dissolution experiments were performed on all ten tropisetron microsphere formulations in HCl buffer at pH 1.2 for two hours, with phosphate buffer at pH 6.8 serving as the dissolution medium for the subsequent ten hours. The findings from the dissolution study indicated that utilizing a greater concentration of sodium alginate reduced the controlled release profile of the drug, achieving maximum release in 8 to 9 hours, while a higher concentration of ethyl cellulose facilitated a slower drug release owing to its hydrophobic nature. Employing a sufficient proportion of all three polymers yields an ideal controlled release effect for up to 12 hours, particularly observed in the TM10 formulation containing 30% of

the combined polymers. Figure 5 presents the comparative *in vitro* drug release data for formulations TM_1 to TM_{10}.

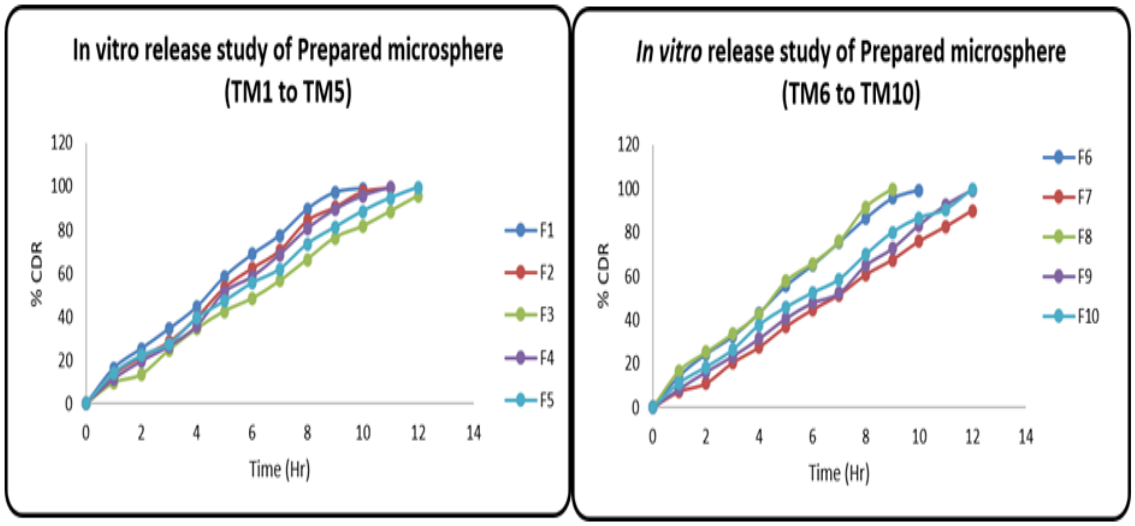

Figure 5: Comparative *in vitro* drug release of prepared tropisetron microspheres

***In Vitro* Release Kinetic Studies of Tropisetron Microspheres**

The computed values for various release kinetics of the optimal Tropisetron Microsphere formulations were determined, with regression values displayed in table 6. The graphical representations for various kinetic analyses are illustrated from figure 6.

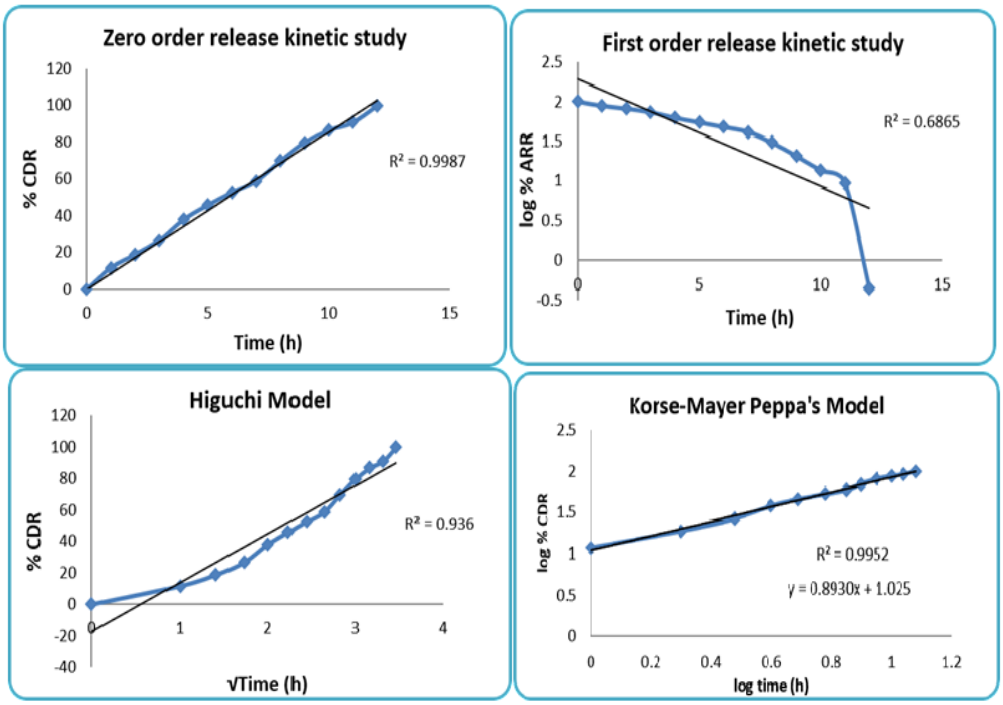

Figure 6: *In vitro* release kinetic study best Tropisetron microspheres (TM_{10})

Table 6: Regression values of *in vitro* release kinetic study best Tropisetron microspheres (TM_{10})

Formulation	R^2value of Zero order	R^2 value of 1st order	R^2value of Higuchi model	R^2value of Peppa's model	'n' value of Peppa's model
TM_{10}	0.9987	0.6865	0.9361	0.9852	0.8930

The *in vitro* drug release data from the optimal tropisetron microsphere formulations (TM_{10}) were analyzed using several kinetic models, and regression coefficients were determined. The optimal formulation exhibited somewhat straight zero-order plots, as seen by their superior regression values. The release exponent 'n' for optimum formulations was determined to range between 0.5 and 1 ($0.5<n<1$), suggesting a combination of diffusion and erosion mechanisms, referred to as anomalous diffusion. In the current investigation, the *in vitro* drug release kinetics of the optimal formulation adhered to a zero-order release kinetic model, with the drug release mechanism characterized by anomalous diffusion combined with erosion.

Stability Studies

The optimal formulation TM_{10} underwent stability assessments at 40°C and 75% relative humidity for a duration of three months. The efficacy of the formulated microspheres remained between 90% and 100% under accelerated stability conditions. There was no alteration in physical appearance, and it remained chemically stable for three months. The information is displayed in table 7.

Table 7: Stability Study for TM_{10} Formulation

Sl. No	Formulation	Before storage (%)	Stored at 40°C±2°C and 75%±5% RH		
			1st month (%)	2nd month (%)	3rd month (%)
1	TM_{10}	98.75±0.65	97.53±0.45	95.52±0.52	93.46±0.36

CONCLUSION:

The microspheres of Tropisetron, composed of ethyl-cellulose, sodium alginate, and HPMCK15, were effectively fabricated using the ionic gelation process, which was validated as the optimal approach due to its superior percentage yield. The formulation TM_{10} possesses the greatest milligram quantity of drug content, succeeded by the other formulations. The dimensions of a microsphere were assessed using optical microscopy, revealing a consistent size distribution across all batches. The fabricated microspheres exhibited excellent spherical morphology with a smooth surface, as demonstrated by optical microscopy. The in vitro dissolution analyses indicated that the tropisetron microspheres formulation TM_{10} exhibited a superior sustained effect for 12 hours. The dissolution study indicated that utilizing a higher concentration of sodium alginate resulted in a diminished controlled release profile of the drug, with the maximum release occurring within 8 to 9 hours; conversely, an increased concentration of ethyl cellulose led to a slower drug release due to its hydrophobic characteristics. The TM_{10} formulation, which comprises 30% of each of the three polymers, exhibits an optimal controlled release effect lasting up to 12 hours. The release mechanism was ascertained by applying the release data to multiple kinetic equations, including zero-order, first-order, Higuchi, and Korsemeyer-Peppas, and calculating the R2 values for the release profiles of each model. It was determined that with an increase in polymer concentration, the density of the polymer also rises, leading to an extended diffusion path length that the drug molecules must navigate, thus prolonging the drug release of the F10 formulation compared to other formulations. In the current investigation, the in vitro drug release kinetics of the optimal formulation adhered to a zero-order release kinetic model, with the drug release mechanism characterized by anomalous diffusion in conjunction with erosion. The findings of the present study unequivocally demonstrate the promising potential of Tropisetron microspheres as an alternative to traditional dosage forms, as they improve the bioavailability of Tropisetron by circumventing first-pass metabolism and providing a sustained release effect for chemotherapy-induced nausea and vomiting. Nonetheless, additional clinical investigations are required to evaluate the effectiveness of this system for individuals experiencing nausea and vomiting.

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