



Formulation and Evaluation of Posaconazole Microspheres

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ABSTRACT

Sustained release microspheres of Posaconazole drug were prepared by using one of the best methods of preparation of microspheres i.e Ionotropic gelation technique, using various polymers such as Sodium alginate, HPMC, Eudragit, and Carbopol 934 as release rate controlling polymers. The absorption of the antifungal drug Posaconazole is enhanced by gastric acidity of the stomach because the drug shows maximum solubility at acidic pH. hence it was an idea to prepare a sustained-release product which can be given by oral route and may overcome the side effects related with conventional dosage forms. The surface images of the sustained release microspheres were characterized by scanning electron microscopy. The prepared microspheres were characterized by their micromeritics properties such as particle shape and size. Dissolution data obtained from in vitro release studies fitted the Higuchi and Peppas model, and recommends the drug release mechanism as non-fiction diffusion.

Keywords: Posaconazole, FTIR studies, Polymers, Ionotropic gelation technique, SEM analysis, In vitro drug release studies

INTRODUCTION

Microspheres are carrier drug delivery system which plays an important role in micro-particulate novel drug delivery system. Microspheres have been explored extensively for their use in the field of drug delivery and various polymers have been utilized for the formulation of the microspheres, which in turn have been assessed for different purposes. Microspheres are one of the multiple unit dosage forms.¹ eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained. Microspheres are potential drug delivery carrier systems in the segment of novel drug delivery and are prepared using assorted polymers.² Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 μ m.³ They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats, and waxes. Posaconazole is a broad spectrum triazole antifungal agent which is derived from fluconazole.⁴ It disrupts the fungal cell membrane by inhibiting 14 α -methyl group of lanosterol which is an important step in the synthesis of ergosterol.⁵ The current research will be oriented towards the formulation and evaluation of polymeric microspheres of Posaconazole and investigate the release profile of such drugs using natural and synthetic polymers.⁶ To study the effect of the concentration of polymer and calcium chloride on the release profile of Posaconazole. The main objective of this research work is to formulate sustained release microspheres of posaconazole to reduce dosing frequency and to improve patient compliance.

MATERIALS

Posaconazole was obtained from Hetero labs, HYD. Sodium alginate, HPMC, Eudragit, and carbopol 934 were procured from SD Fine Chemicals, Mumbai. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY

FTIR Spectroscopic Study⁷

Fourier Transform Infrared (FTIR) spectroscopy was performed on IR- Prestige-21, Shimatzu make, Japan. FTIR study was carried on Etodolac, Gum Katira and Microsphere showing their characteristic peaks in the region from 400-4000cm⁻¹.

Preparation and evaluation of Posaconazole Microspheres

Selection of polymers for preparation of Microspheres⁸

Polymers were used as excipients for drug formulations and cellulose derivatives are also used for the formulation of Microspheres. In the present study, for the preparation of Microspheres of Posaconazole.

Preparation method of Microspheres⁹

Microspheres containing Posaconazole as a core material were prepared by Ionotropic gelation technique.

Polymers and CaCl₂ was dissolved in 100 mL of deionized water at a concentration corresponding to the formula and 100 mg of Posaconazole was dissolved in 100 mL of polymeric solution. The co-solution was then introduced into the aerosolization nozzle and sprayed into 100 mL of CaCl₂ solution which was continuously agitated with a magnetic stirrer at a speed of 1,000 rpm. After 30 min, microspheres were collected by means of centrifugation at 2,500 rpm and washed three times with distilled water at room temperature.

Formulation table:

Table-: Preparation of Posaconazole Microspheres

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Posaconazole	100	100	100	100	100	100	100	100	100	100	100	100
Sodium alginate	500	500	500	500	500	500	500	500	500	500	500	500
HPMC	100	200	300	400	-	-	-	-	-	-	-	-
Eudragit	-	-	-	-	-	200	300	400	-	-	-	-
Carbopol 934	-	-	-	-	-	-	-	-	100	200	300	400



Evaluation of Microspheres

The prepared Microspheres were evaluated for various parameters such as yield, particle size, drug entrapment efficiency, evaluation of in vitro drug and the effect of different formulation and process variables such as drug to polymer ratio, type of polymer, speed, and combination of polymers were studied.

Percentage yield¹⁰

The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components that were used for the preparation of the microspheres.

% Yield = Actual weight of microspheres/Total weight of drug and polymer ×100

Scanning Electron Microscope (SEM)¹¹

Studies were carried out by using JEOL MAKE (UK), (MODEL-JSM6360). Microspheres were mounted on conducting stubs using double-sided adhesive tape and vacuum coated with gold palladium film using a sputter coater (Edward S-150, UK). Images were taken using 17 kV electron beam intensity in a scanning electron microscope to examine the surface morphology of the samples.

Particle size determination¹²

Optical microscope was used to determine the size of the microspheres. This method involves the calibration of eyepiece micrometer for which the stage micrometer is used. In stage micrometer one mm is divided into 100 equal divisions and hence, each division is equal to 10 μ m and the particles are measured chosen fixed line across the Center of the particle. The average diameter of was calculated using following formula.

$$\text{Average diameter} = \Sigma nd/n \times C.F.$$

Where n = number of microspheres, d =diameter of microspheres, C.F =calibration factor

Drug entrapment efficiency (DEE)¹³

The amount of drug entrapped was estimated by crushing 50 mg of Microspheres using mortar and pestle, and extracting drug with aliquots of 7.4 pH buffer repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 7.4 pH buffer. The solution was taken in a beaker and sonicated in a bath sonicator for 2 hours. The solution was filtered and absorbance was measured after suitable dilutions spectrophotometrically at 292 nm against an appropriate blank.

The amount of drug entrapped in the Microspheres was calculated using the following formula –

$$\text{DEE} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100$$

Swelling index¹⁴

Degree of swelling is expressed as the percentage of water in the hydrogel at any instant during swelling. Swell ability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. The in-vitro swelling property of microspheres was studied in Phosphate buffer pH 7.4. It can be concluded from the data that with an increase in polymer concentration, the degree of swelling also increases. Thus we can say that amount of polymer directly affects the degree of swelling.

In vitro drug release study¹⁵

The microspheres equivalent to 20 mg of drug posaconazole were filled in hard gelatin capsules (no. 4) and coated with 1% w/v solution of cellulose acetate phthalate (CAP) by dip-coating method. The coating was accomplished by dipping the filled capsules thrice in CAP solution and air drying after each coating step successively. In-vitro drug release on the capsules size 4 was undertaken using USP Apparatus 2 at 50 rpm, in 900 ml of medium at 37°C \pm 0.5°C with a wire sinker. For the enteric capsules 2 h of exposure in 0.1 N hydrochloric acid (pH 1.2) followed by testing in phosphate buffer of pH 7.4 for 10 h. A suitable volume of sample was withdrawn of after suitable time interval and equal volume of fresh medium was replaced to maintain a constant total volume. Samples were filtered using 0.45 μ m filter. Rabeprazole sodium concentrations were determined by UV spectrophotometer (Jasco-V-530) at a wavelength of 292 nm.

Drug release kinetics¹⁶

The models used were zero order (equation 1) First order (equation 2) and Higuchi model (equation 3) and Koresmeyer Peppas model (equation 4).

i) zero order kinetics:

$$R = K_0 t \quad \text{-- (1)}$$

R=cumulative percent drug

K₀=zero order rate constant

ii) First order kinetics

$$\log C = \log C_0 - K_1 t / 2.303 \quad \text{-- (2)}$$

where C = cumulative percent drug

K₁ = first order rate constant

iii) Higuchi model

$$R = K_H t^{0.5} \quad \text{-- (3)}$$

Where R = cumulative percent drug

K_H = Higuchi model rate constant

iv) Korsmeyer Peppas model:

$$M_t / M_\infty = K_k t^n$$

$$\log M_t / M_\infty = \log K_k + n \log t \quad \text{-- (4)}$$

where K_k = Korsmeyer Peppas rate constant

' M_t / M_∞ ' is the fractional drug, n = diffusional exponent, which characterizes the mechanism of drug (Simon Benita, 2007).

Diffusional exponent (n)	Drug mechanism
0.43	-- Fickian diffusion
0.43- 0.85	-- Anomalous (non- Fickian) transport
0.85- 1	-- Case II transport
> 1	-- Supercase II transport

The obtained regression co-efficient (which neared 0.999) was used to understand the pattern of the drug from the Microspheres.

Stability studies¹⁷

The success of an effective formulation can be evaluated only through stability studies. The prepared Posaconazole Microspheres placed on plastic tubes containing desiccant and stored at ambient conditions for a period of 3 months.

RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected polymers and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-polymer mixture, which confirmed the absence of any chemical interaction between the drug, polymer, and other chemicals.

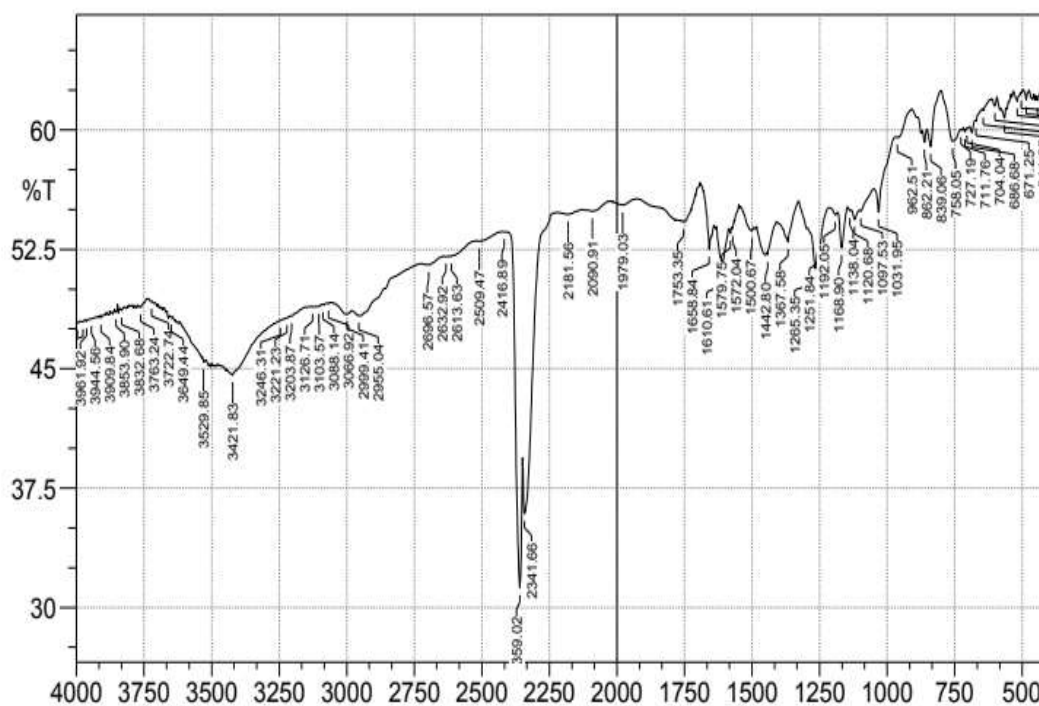


Fig.:- FTIR Studies of Posaconazole

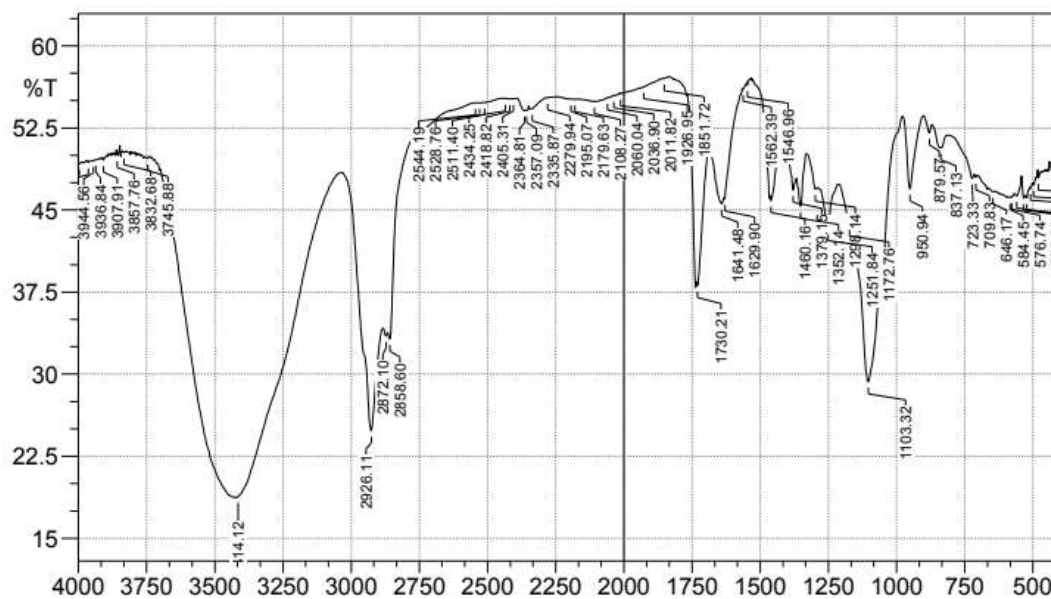


Fig:- FTIR Studies of optimized formulation

EVALUATION STUDIES

Table:- Characterization of Yield of microspheres, Drug entrapment efficiency

F. code	% Yield	Drug entrapment efficiency	Particle size (nm)
F1	72.21	82.35	726
F2	68.5	80.21	729
F3	66.87	79.88	720
F4	71.28	81.20	738
F5	65.54	80.33	624
F6	64.32	78.25	608
F7	70.28	81.39	497
F8	75.34	80.88	594
F9	68.82	77.54	548
F10	76.38	84.96	638
F11	70.40	79.30	728
F12	73.69	82.32	689

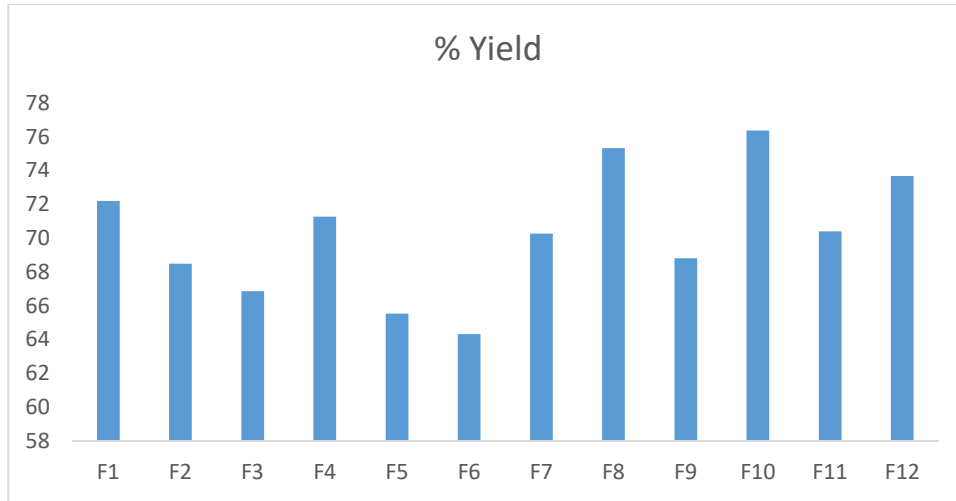


Fig:- % Yield of all formulations

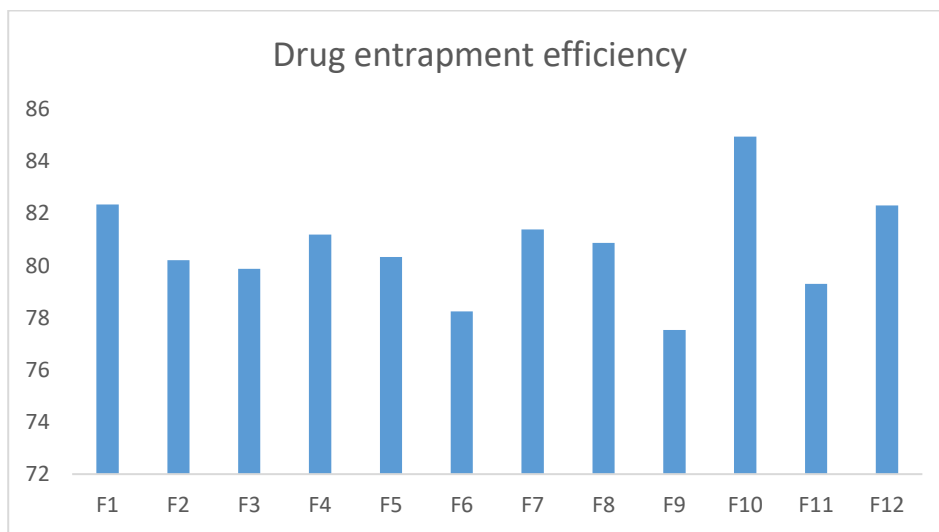


Fig:- Drug entrapment efficiency of all formulations

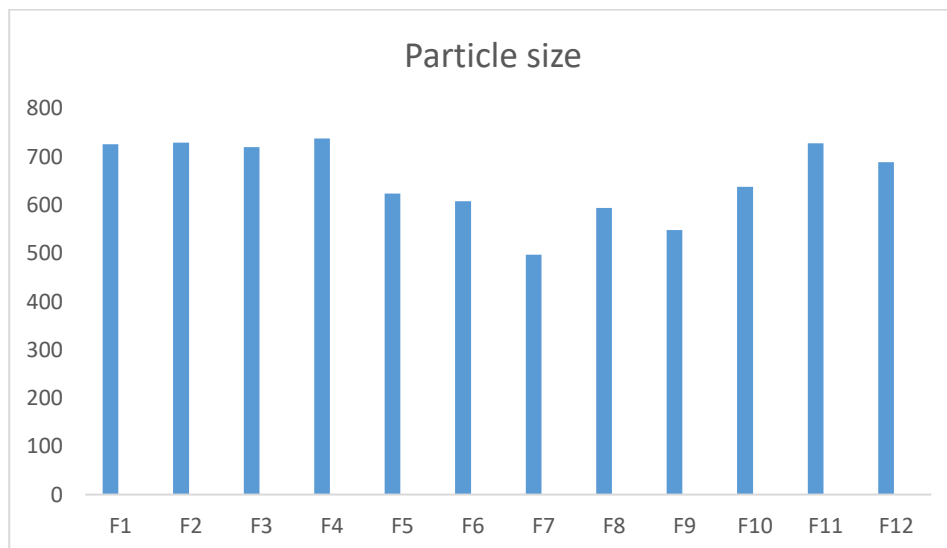


Fig:- Particle size of all formulations

SEM Analysis

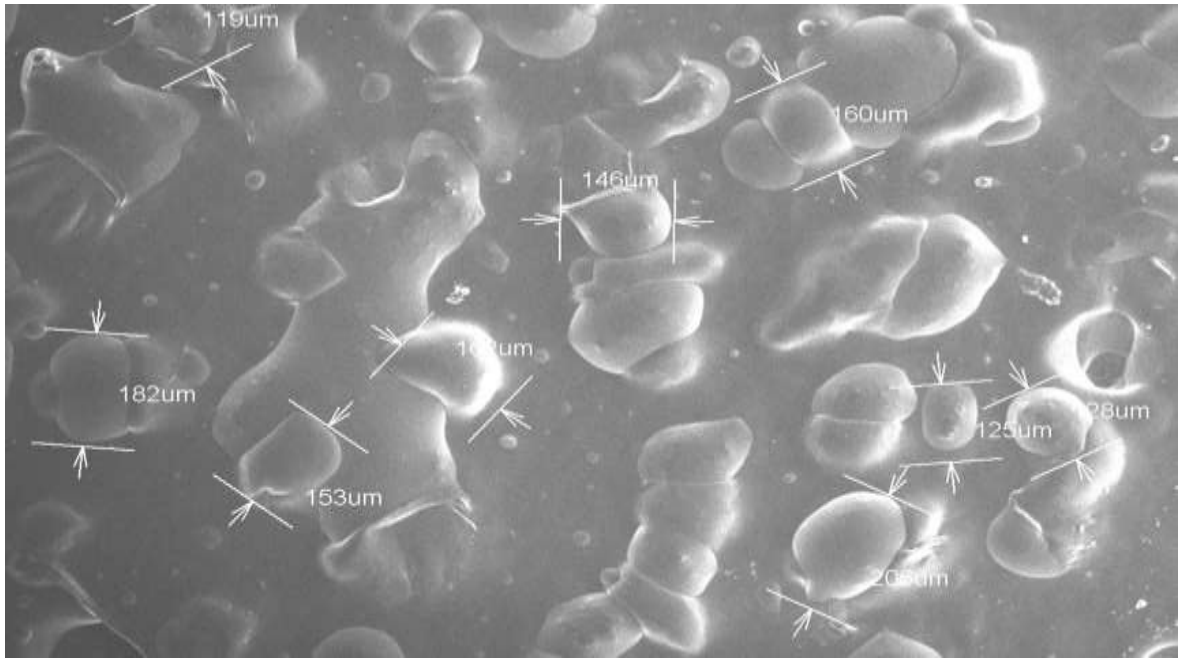


Fig:- SEM photograph of Diclofenac microspheres at 100x and 1000x magnification.

Particle size

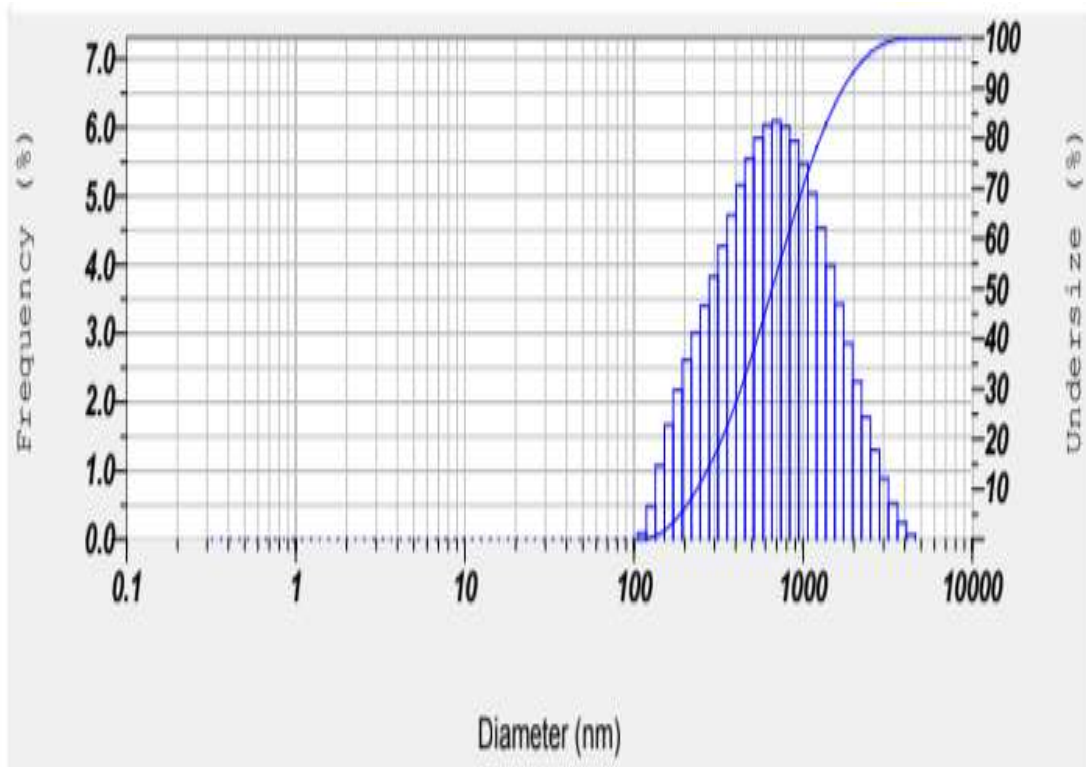


Fig:- Particle size of optimized formulation

Drug release studies

Table-: Drug release studies all formulations

TIME (hours)	F1	F 2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	8.52	9.35	8.25	9.56	9.20	9.52	10.23	9.35	9.89	10.20	10.11	9.99
2	12.93	14.14	16.38	17.93	18.34	17.93	16.90	18.12	18.25	19.20	19.02	18.96
3	19.56	20.37	24.30	23.65	21.21	25.38	27.98	26.94	28.25	28.67	26.90	27.12
4	32.45	35.96	36.98	34.14	31.95	30.52	32.68	33.47	35.67	36.95	35.28	34.25
5	40.19	43.58	40.12	40.21	42.38	43.89	45.96	43.86	42.17	48.91	42.18	45.24
6	50.25	49.68	50.34	49.58	47.93	50.12	49.84	50.12	51.25	54.33	50.93	49.83
7	57.16	58.94	55.98	53.28	50.32	55.96	53.80	55.90	59.38	58.92	55.68	54.25
8	62.20	61.25	59.31	60.17	59.82	60.21	62.25	60.93	63.19	63.56	61.19	60.17
9	63.49	65.89	66.30	60.14	62.98	67.89	68.92	69.86	65.17	73.82	64.25	65.80
10	75.68	77.35	80.21	78.25	72.14	78.54	73.48	75.14	76.91	82.25	81.25	79.63
11	80.29	81.67	85.35	86.92	86.79	87.93	88.25	84.36	89.93	90.25	89.63	88.32
12	93.56	90.21	92.67	91.20	90.25	92.37	93.54	91.22	95.32	96.38	95.37	94.58

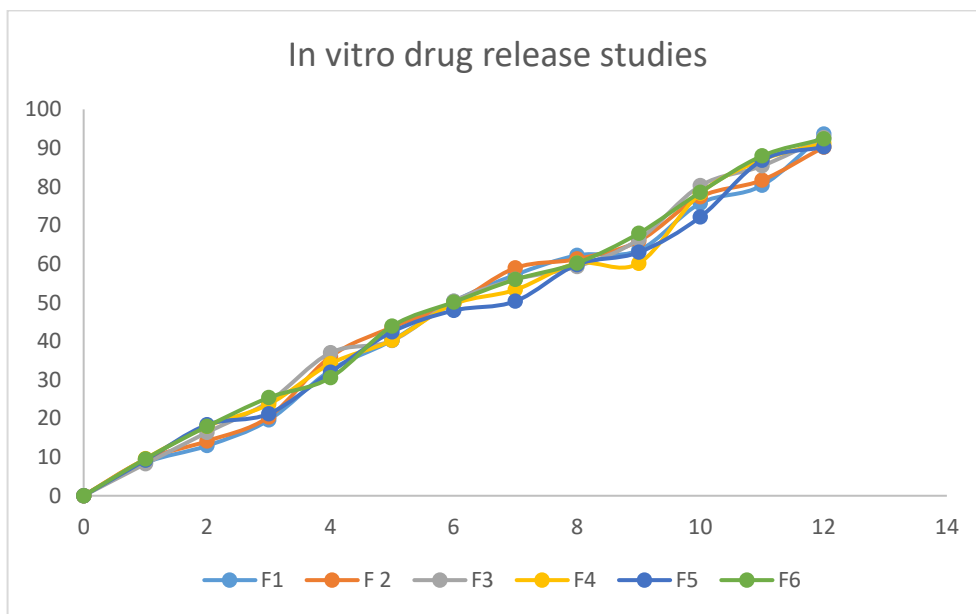


Fig-: In vitro drug release studies of (F1-F6) formulation

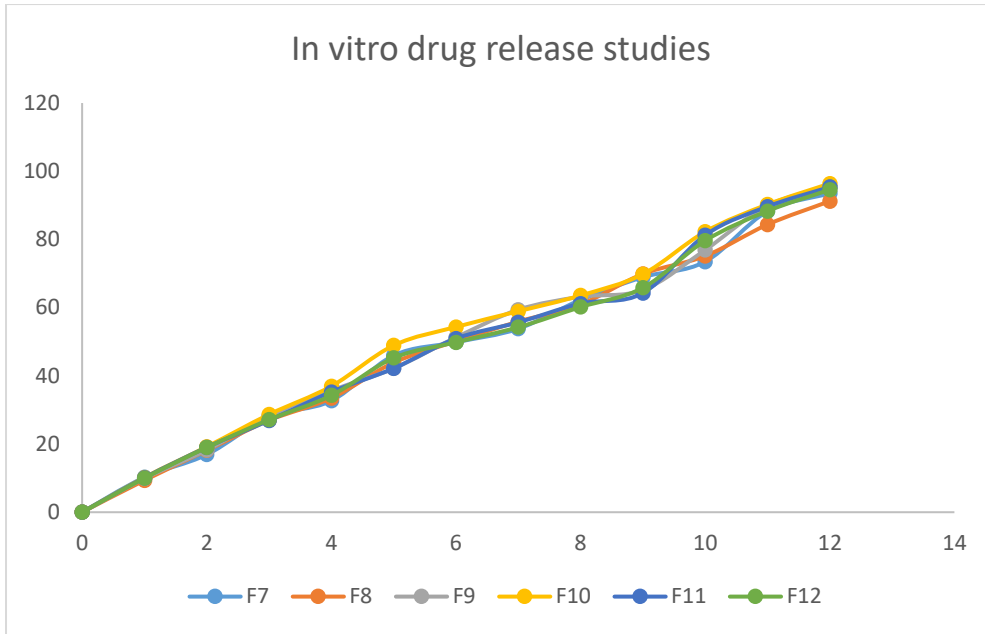


Fig:- In vitro drug release studies of (F7-F12) formulation

Drug release kinetics

Zero order kinetics:

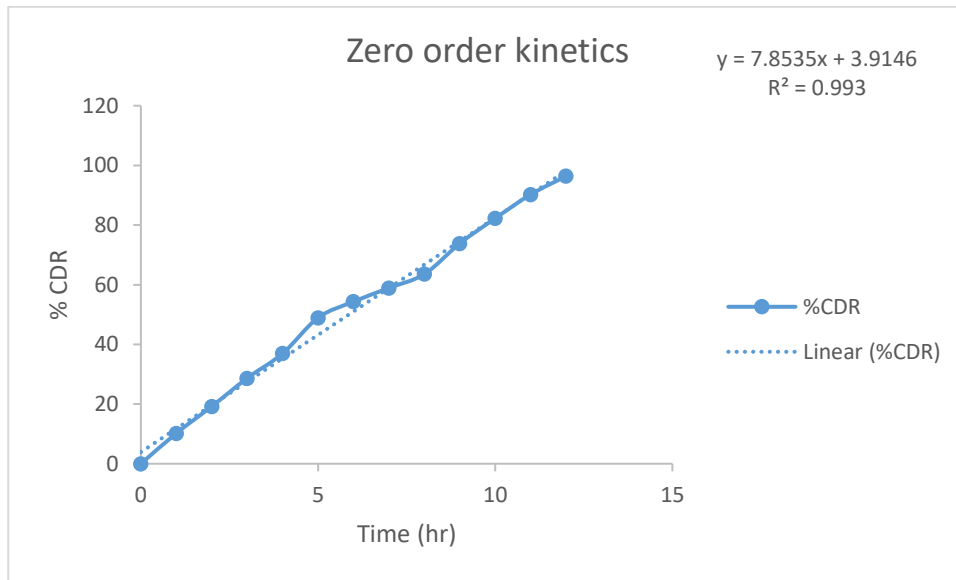


Fig:- Zero order plot for optimized formulation

First order kinetics

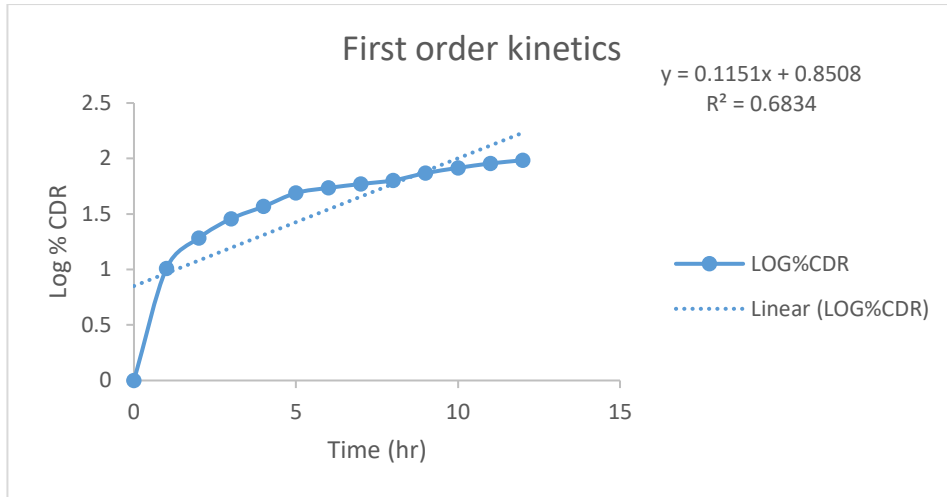


Fig-: First order for optimized formulation

Higuchi model

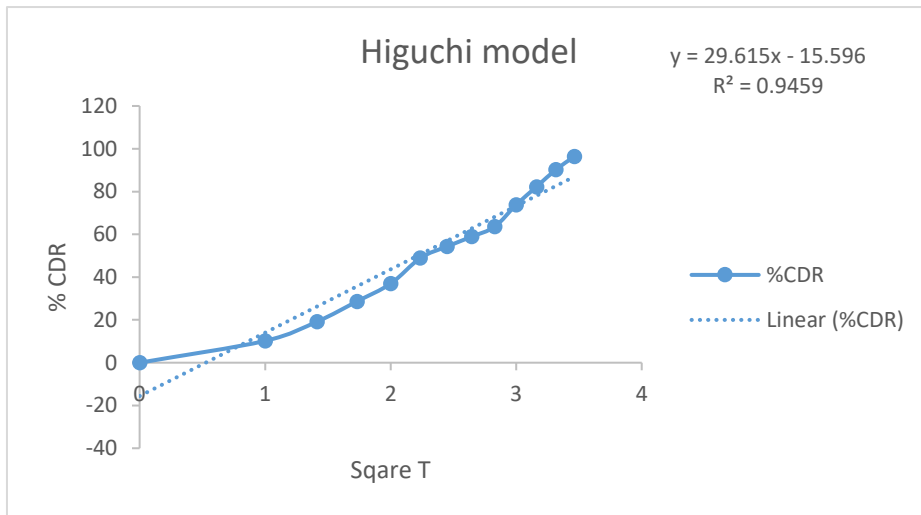


Fig-: Higuchi plot for optimized formula

Korsmeyer peppas

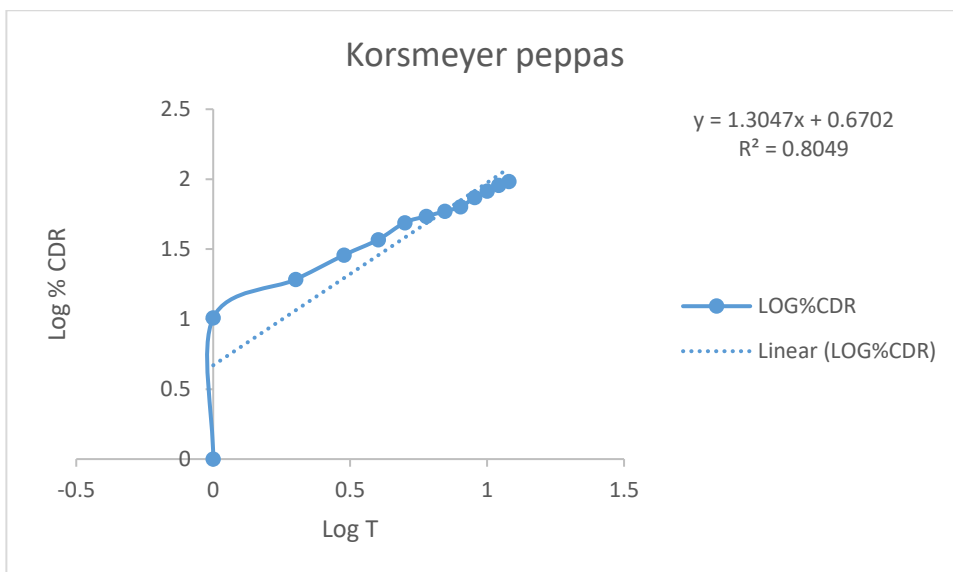


Fig-: Korsmeyer peppas plot for optimized formula

Stability studies

There was no significant change in physical and chemical properties of the Microspheres optimized formulation after 90 days. Parameters quantified at various time intervals were shown;

Table-: Results of stability studies of optimized formulation

F. Code	Parameters	Initial	1 st Month	2 nd Month	3 rd Month	Limits as per Specifications
F-10	25°C/60%RH % Release	96.38	96.12	95.89	94.67	Not less than 85 %
F-10	30°C/75% RH % Release	96.38	96.06	95.82	96.58	Not less than 85 %
F-10	40°C/75% RH % Release	96.38	96.01	95.76	95.42	Not less than 85 %

CONCLUSION

Attempt have been made to prepare sustained release microspheres of Posaconazole, These microspheres are used to treatment of fungal infections. The microspheres were prepared by Iontropic gelation technique method using natural polymers and synthetic as retarding polymers and evaluated for parameters like percentage yield, particle size, entrapment efficiency, and the effect of preparation and process variables such as drug-polymer ratio, speed, type of polymer and combination of polymers on evaluated parameters. Microsphere morphology was evaluated by SEM. The yield and entrapment efficiency were high for Sodium alginate with Carbopol 934 microspheres Particle size, entrapment efficiency, and production yield were influenced by the type of polymer, polymer concentration, stirring speed, and combination of polymers. *In vitro* dissolution of optimized formulations of various Polymer in pH 7.4 formulations are releasing the drug up to 8 hrs.

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