IJBPAS, November, 2025, 14(11): 5814-5823

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF FLOATING MICROSPHERES OF TINIDAZOLE

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Received 15th July 2024; Revised 20th Sept. 2024; Accepted 21st Oct. 2024; Available online 1st Nov. 2025

https://doi.org/10.31032/IJBPAS/2025/14.11.9496

ABSTRACT

The objective of the present study was to formulate and optimize floating microspheres of tinidazole. Tinidazole is a BCS class II drug i.e. low solubility and high permeability. The improve solubility of drug using inclusion complex by kneading method with help of β-CD polymer 1.1 ratio. To achieve these objective nine formulations of microspheres were prepared by congealable disperse phase encapsulation method. A 3^2 factorial design was employed in formulating the microspheres with Ethyl cellulose and Eudragit S-100 as independent variables. Drug entrapment and Percentages drug release was considered as dependent variable. The proposed floating system was evaluated by preliminary evaluation parameters, micrometric investigation as well as percentage yield of microsphere, Drug entrapment efficiency, Drug content, Floating behaviour, In vitro drug release studies and their kinetics. IR and DSC study confirmed the drug polymer compatibility and scanning electron microscopy indicates that the microspheres were spherical in shape and rough surface. Amongst M4 was found to be the best formulation as it releases Tinidazole 90.26±0.65 in a sustained manner. Formulated microspheres prolonged the drug release by up to 12 hrs.

Keywords: Tinidazole, Microspheres, Floating System, Drug entrapment

1. INTRODUCTION:

Oral route is the most convenient and extensively used route for drug administration. Over the years the oral dosage forms have become sophisticated with development of sustained release drug delivery system (SRDDS).

Gastroretentive dosage forms significantly extend the period of time, over which drug may be released and thus prolong dosing intervals and increase patient compliance. These systems can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract, thus ensuring optimal bioavailability.

Tinidazole is a synthetic antiprotozoal and antibacterial agent. That belongs to the nitroimidazole class. It is 1-[2-(ethylsulfonyl) ethyl] -2-methyl-5-nitroimidazole. Tinidazole is effective therapy against protozoa such as *Trichomonasvaginalis, amoebiasis,* and *giardiasis*. Tinidazole is one of the most effective drugs available against anaerobic bacterial infection. Tinidazole is amebicidal, bactericidal, and trichomonicidal. Reduced it then disrupts DNA helical structure, thereby inhibiting bacterial nucleic acid synthesis. This eventually results in bacterial cell death **[1]**.

2. MATERIAL AND METHODS:

2.1 Materials:

Tinidazole was received as a gift sample from Shalina Laboratories Pvt. Ltd Jejuri, Pune. Beta cyclodextrin and Ethyl cellulose were of SISCO CHEM. Lab., Mumbai and all other excipients used were of Research Lab. Fine Chem., Mumbai.

2.2 Methods:

2.2.1 Preparation of inclusion complex by kneading method:

TNZ and β-CD were blended for 30 min in 1:1 molar ratio by wetting with appropriate quantity of ethanol to form a paste. After that, it was dried overnight at 40°C in oven. The mass was pulverized and passed through sieve 100 **[2]**.

2.2.2 Floating Gastroretentative Microspheres Preparation:

The Floating Gastroretentative Microspheres was prepared by using Congealable Disperse Phase Encapsulation Method. The external phase was prepared as a 200 ml of a 0.25% w/v solution of sodium lauryl sulphate (SLS) in de-ionized water. This aqueous phase was heated to about 80°C (temperature higher than the melting point of wax). It was simultaneously stirred at 1000 rpm using a mechanical lab stirrer. 2 gm of white beeswax and 0.05 gm of stearyl alcohol were weighed into a porcelain crucible and melted over a water bath. 1gm of Tinidazole was weighed and add ethyl cellulose and eudragit S-100 stirred into the molten wax phase. This one phase melt was added to the heated aqueous phase in a drop wise manner while stirring at 750 rpm for 2 minutes followed by stirring at 600 rpm for 3 minutes. After 5 minutes, the oil-in-water emulsion was rapidly cooled pouring into about 400 ml of ice-cold water (about 4°C) and stirring was continued as the emulsion cooled. The microspheres were collected by

filtration through Whatman #1 filter paper. The microspheres were washed with about 100 ml of water were used for washing the microspheres to remove any traces of drug or surfactant residues shown in **Table 1 [3]**.

3. Full factorial design:

The experiments were performed with a 3^2 randomized full factorial design. In this study two factors were evaluated each at three levels, and experimental trials were performed at all nine possible combinations. The amounts of Ethyl cellulose (X1) and Eudragit S-100 (X2) were selected as independent variables. The drug entrapment and percentage drug release were selected as dependent variable. The data were evaluated using the Design Expert software (version 13.0.5.0), contour plot and 3D response surface graphs were plotted **[4]**.

Table 1: Formula and composition of batches

Batch code	Drug (mg)	Bees Wax (mg)	Stearyl alcohol (mg)	Ethyl cellulose (mg)	Eudragit S100 (mg)	Sodium lauryl sulphate (mg)	Sodium bicarbonate
M_1	1000	2000	50	100	50	250	300
M_2	1000	2000	50	100	60	250	300
M_3	1000	2000	50	100	70	250	300
M_4	1000	2000	50	200	50	250	300
M_5	1000	2000	50	200	60	250	300
M_6	1000	2000	50	200	70	250	300
M_7	1000	2000	50	300	50	250	300
M_8	1000	2000	50	300	60	250	300
M_9	1000	2000	50	300	70	250	300

(Aqueous Phase used was 100 ml water + SLS)

4. Evaluation:

4.1 Preformulation studies:

4.1.1 Infrared spectroscopy

The IR spectrum of Tinidazole was recorded using Fourier transform infrared spectroscopy to check its purity. The spectrum was recorded over the wave number of 4000 to 400 cm^{-1}.

4.1.2 Differential Scanning Calorimetry (DSC)

DSC was performed to study the thermal behaviour of drug using Mettler Toledo instrument. The samples were heated in hermetically sealed aluminium pans under nitrogen flow (30 ml/min) at a scanning rate of 10◦C/min from 40◦C to 400◦C.

4.2 Evaluation of prepared Microspheres: [5]

4.2.1 Percentage yield:

The prepared microspheres were collected and weighted. The actual weight of obtained microspheres divided by the total amount of all material that was used for the preparation of the microspheres (equation):

% yield: Actual weight of microspheres /Total weight of excipients and drug ×100

4.2.2 Drug entrapment efficiency:

Drug entrapment efficiency was determined by using indirect method. The method

utilizes filtrates of polyelectrolyte solution (i.e. Sodium lauryl sulphate) from each batch. One ml of the filtrate was taken which was further diluted upto 10 ml with 0.1 N HCL and absorbance was taken by UV spectrophotometer at 308.5 nm **[6]**.

4.2.3 Floating behaviour:

The in-vitro floating behaviour of microspheres was studied by using 900 ml 0.1N HCL at 37±0.5 ºC. The speed of rotation was maintained at 50 rpm. The floating lag time (the period between placing microspheres in the medium and buoyancy beginning) and floating duration of microspheres were determined by visual observation.

4.2.4 Drug Content:

Drug content was determined using UV-Spectrophotometer. Drug-loaded microspheres were crushed and 10 mg was suspended in 100 ml 0.1N HCl solution. This suspended solution was kept for 24 hr. It was stirred for 5 min and filtered. Tinidazole content in the filtrate was determined spectrophotometrically at 308.5 nm using a regression derived from the standard curve **[7]**.

4.2.5 In vitro dissolution studies:

In vitro drug release of the samples was carried out using type-II dissolution apparatus (Paddle type). The dissolution medium, 900 ml of 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5ºC and 50 rpm. Microspheres was placed in each vessel and operated the apparatus at 50 rpm for 12h. Withdrawn 5ml of the sample solution from each vessel through 5ml syringes and replaced with equal volume of fresh dissolution medium previously equilibrated to 37 ± 0.5°C. Each sample was filtered through 0.45µm filter and collected in separate vials. The samples were analyzed by UV spectrophotometer at 308.5 nm.

4.2.6 Scanning Electron Microscopy:

The surface morphology of prepared floating microspheres was studies using Scanning Electron Microscopy. The microspheres preparation was scanned randomly, and photography were taken at appropriate magnification **[8]**.

4.2.7 Stability Studies

Short-term stability studies on optimized formulation were carried out by storing the microspheres at 45ºC±2ºC and 75% RH±5% for 3 month. At the end of month, the microspheres were examined for any physical change, changes in in-vitro dissolution studies.

5. RESULTS AND DISCUSSION:

5.1 Preformulation studies:

5.1.1 FTIR analysis

The FTIR spectra of tinidazole showed the following characteristic peak at 3214.47 (cm^{-1}) due to N-H amine stretch. The drug exhibited distinctive peak at 2916.47(cm^{-1}) due to C-H aromatic stretch. The peaks at 1038.06 (cm^{-1}) due to S=O sulfoxide stretch,

peak at 1520.75 (cm^{-1}) due to C=C alkene aromatic stretch, at 1298.97 (cm^{-1}) due to C-N amine stretch. The peak at 3064.18 (cm^{-1}) due to C-H aromatic stretch. Thus, from FTIR spectrum of tinidazole showing **Figure 1** it was found that the observed peak values are in the range of reported peak values.

5.1.2 Differential Scanning Calorimetry:

A sharp endothermic peak was observed in above DSC thermogram at 126.38ºC which indicates crystalline nature and melting of Tinidazole. Such an endothermic peak was also reported for standard drug material near to the melting range. It was concluded that Tinidazole drug was in pure form shown in **Figure 2 [9]**.

5.2 Evaluation of prepared microspheres of Tinidazole:

5.2.1 Percentage yield, Entrapment efficiency, Drug content, floating lag time and total floating time: (Table 2)

The percentage yield of all batch's ranges between 79.42% ± 0.06 to 85.50±0.06. Batch M_7 shows highest percentage yield 85.50±0.06. All formulation batches showed entrapment efficiency ranges from 85.65±0.49 to 91.9±0.15. Batch M4 shows highest entrapment efficiency 91.9±0.15. The drug content of all formulation batches was found to be between 91.57±0.10 to 95.91±0.23. Batch M4 shows highest drug content 95.91±0.23. Here, floating lag time increases and total floating time decreases from M1 to M9 batch, which was due to higher concentration of polymers from M1 to M9. As higher amount of sodium bicarbonate was needed to lift higher amount of polymers. The results of percentage yield, drug entrapment, drug content, floating lag time and total floating time were shown in **Table 2 [12]**.

5.2.2 In vitro dissolution study:

In vitro drug release studies of Tinidazole floating microspheres were performed in 0.1 N HCL in dissolution test apparatus. M1 to M9 show percentage drug release 75.65±1.17 to 90.26±0.65 at end of 12 hour shown in **Figure 3**. Amongst M4 was found to be the best formulation as it sreleases Tinidazole 90.26±0.65 in a sustained manner.

5.2.3 Kinetics of Drug Release:

The in-vitro dissolution data were analyzed by different kinetic models in order to find out the best fitted model. The values of correlation (R^2) were calculated. The n-value i.e. diffusion exponent was also calculated which describes the drug release mechanism.

From experimental data obtained from the in vitro drug release were fitted into Higuchi model, The interpretation of these data was based on the value of regression coefficient. The in vitro drug obtained shown in **Figure 4** the highest regression coefficient value for Higuchi model that is 0.9831, thus indicating diffusion of the drug from the

polymer matrix considered to be predominant mechanism of drug release **[13]**.

5.3 Factorial model and Contour Plot and 3D Response Surface Plot:

ANOVA results indicated that concentration of ethyl cellulose and Eudragit S-100 showed effect on % drug entrapment and % drug release. The model F-value of 9.29 and 9.20 with probability P > F of 0.05 implies that this model is significant with only a 4.80% and 4.86% chance that this F value could have occurred due to noise. **Figure 5** shows the contour plot of effect of ethyl cellulose on % drug entrapment. It represented that when the concentration of ethyl cellulose increases then drug entrapment was found to be increasing in a microsphere. **Figure 5** shows the resulting response surface plot for % drug entrapment. It is demonstrated that the % drug entrapment depends on the concentration of ethyl cellulose. The highest drug entrapment was obtained at optimum level of ethyl cellulose. **Figure 6** shows the contour plot of effect of eudragit S-100 on % drug release. It represented that when the concentration of eudragit S100 increases then drug release was found to be increasing in a microsphere. **Figure 6** shows the resulting response surface plot for % drug release. It is demonstrated that the % drug release depends on the concentration of eudragit S-100. The highest drug release was obtained at optimum level of eudragit S-100 **[10, 11]**.

5.4. Scanning electron microscopy:

Figure 7 shows the appearance of surface morphology of the Drug loaded microspheres using scanning electron microscopy which indicates microspheres were spherical in shape with slightly rough surface. There is no external formation of drug crystals **[14]**.

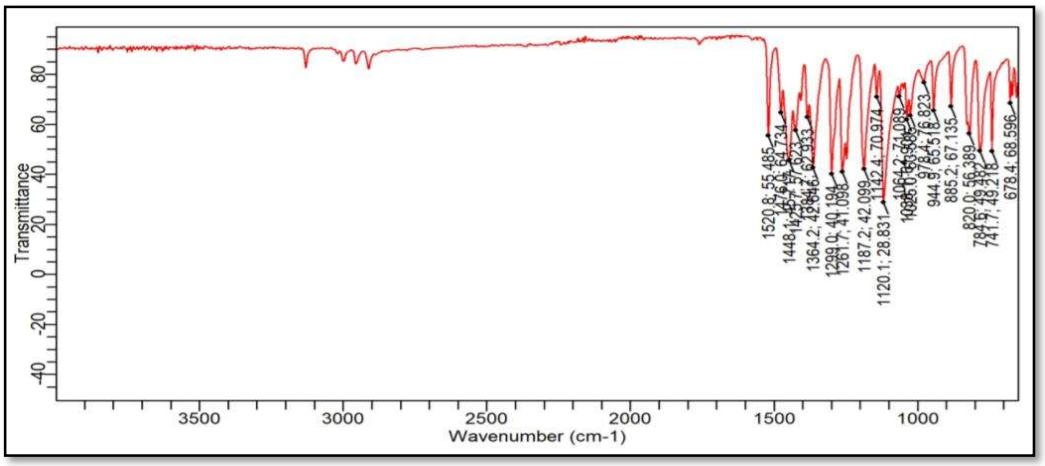

Figure 1: FTIR spectra of Tinidazole

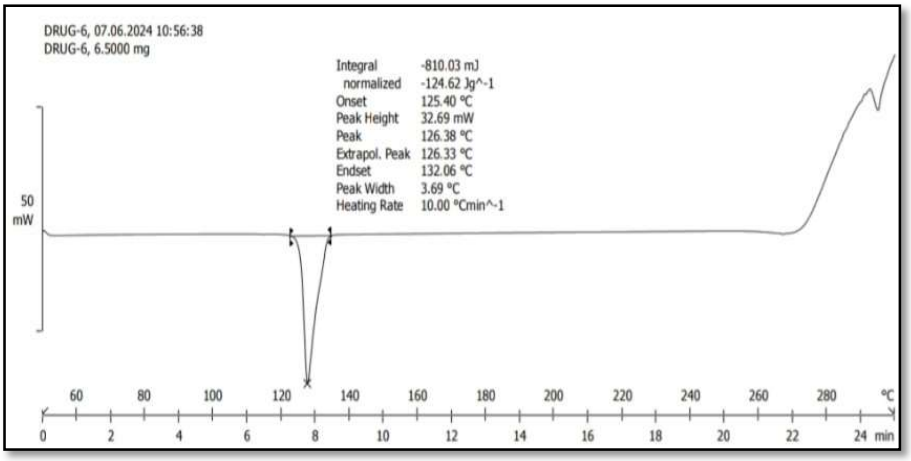

Figure 2: DSC of Tinidazole

Table 2: Evaluation parameters of floating microspheres of Tinidazole

Sr. no.	Batch code	Percentage yield (%)	Entrapment efficiency	Drug Content	Floating lag time (sec)	Total floating time (Hrs.)
1	M_1	79.71±0.04	88.1±0.40	91.98±0.37	5±0.06	12 hr.
2	M_2	81.15±0.07	90.32±0.30	94.27±0.31	4±0.01	12 hr.
3	M_3	82.60±0.06	89.1±0.25	91.57±0.10	6±0.06	12 hr.
4	M_4	80.57±0.07	91.9±0.15	94.61±0.09	4±0.03	12 hr.
5	M_5	79.42±0.06	88.9±0.30	95.69±0.23	5±0.07	12 hr.
6	M_6	81.44±0.06	89.23±0.45	95.91±0.23	4±0.07	12 hr.
7	M_7	85.50±0.06	90.5±0.55	92.50±0.12	6±0.04	12 hr.
8	M_8	80.28±0.04	85.65±0.49	93.74±0.31	5±0.05	12 hr.
9	M_9	83.47±0.05	87.4±0.32	92.91±0.37	7±0.03	12 hr.

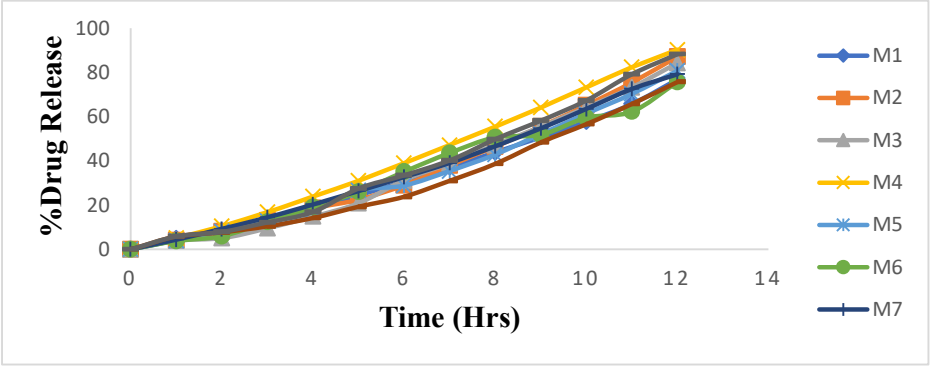

Figure 3: In-vitro dissolution profile of formulation

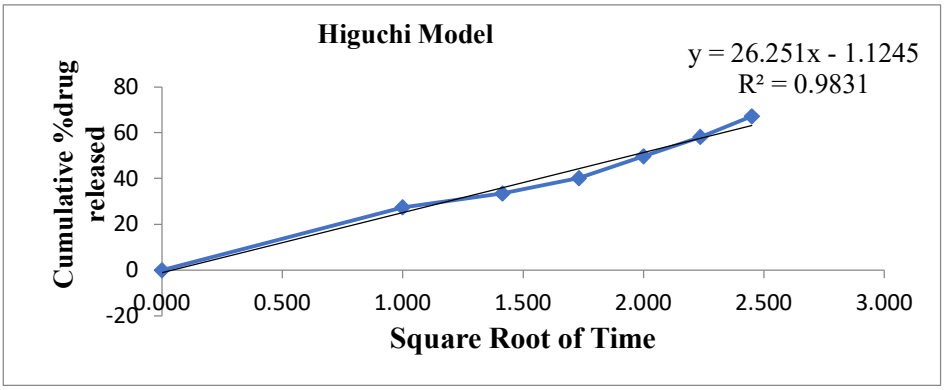

Figure 4: Higuchi model kinetics of M4 batch

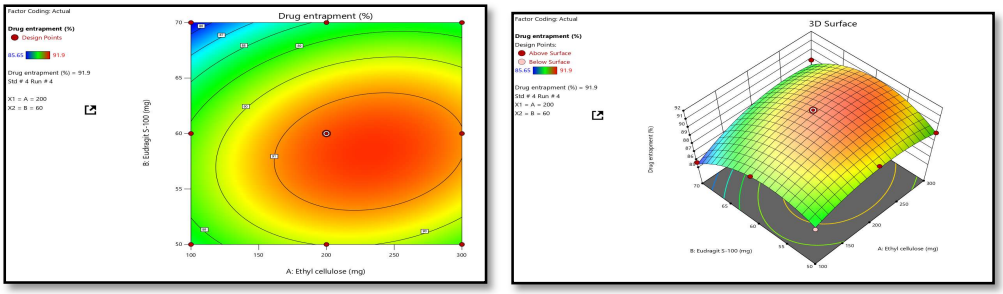

Figure 5: Contour plot and 3D Response Surface plot show for % Drug Entrapment

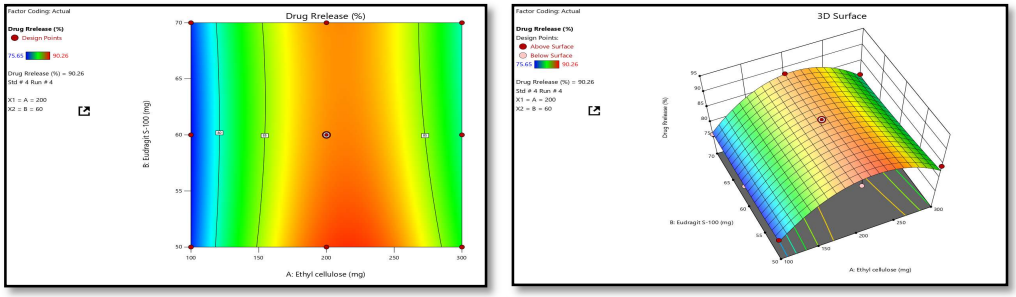

Figure 6: Contour plot and 3D Response Surface plot show for % Drug Release

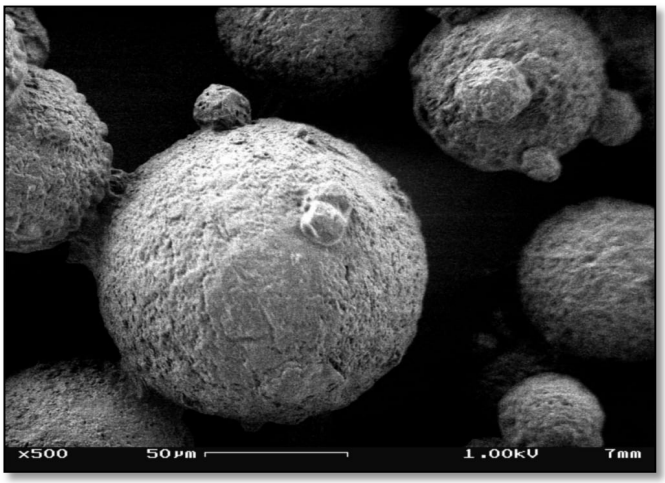

Figure 7: Scanning Electron Microphotographs of Drug loaded microspheres

5.5 Stability studies:

Short-term stability studies of the optimized formulation indicated that there were no significant changes in physical appearance, drug content, drug entrapment and in vitro dissolution studies at the end of three-month period. The optimized formulation did not show any significant change in cumulative % drug release after 12 hr, drug content, drug entrapment when kept at normal condition **[15]**.

CONCLUSION:

The Tinidazole floating microspheres were successfully formulated by congealable disperse phase encapsulation method. FTIR and DSC studies indicated that there were no drug excipient interactions. Tinidazole is a BCS class II drug i.e. low solubility and high

permeability. To enhance solubility of drug using inclusion complex by kneading method with the help of β-CD. 3^2 full factorial design was applied successfully. From all possible nine batches M4 batch was selected as optimized batch on the basis of drug entrapment, drug content and drug release in sustained manner. Formulation M4 was optimized due to its ability to sustained the drug released over the period of 12 hrs. It showed 90% drug release at the end of 12 hrs. The Higuchi model is best fitted model. Surface morphology of the drug loaded microspheres using scanning electron microscopy which indicates microspheres were spherical in shape with slightly rough surface. Stability test was carried out at 45ºC±2ºC and 75% RH±5% temperature. The stability study was performed for 3 months. It was observed that the best batch was stable till 3 months.

ACKNOWLEDGEMENT:

Authors are thankful to Navsahyadri Institute of Pharmacy, Pune, for providing necessary facilities to carry out this work. Would like to thank our guide Mrs. Trushali. A. Mandhare and Miss Pooja Kashid who took his valuable time to guide us throughout the process.

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