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# Formulation and Evaluation of Microbially Activated Osmotic Drug Delivery System for Colon Targeted Tinidazole Tablets

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## ABSTRACT

The Microbially Activated Osmotic drugs Delivery for Colon targeted Tinidazole Tablets were developed. The developed systems consisted of osmotic core (drug, osmotic agent and wicking agent), coated with semi permeable membrane (SPM) containing Guar gum as pore former, coated core were then further coated with enteric coating to protect the system from acidic environment of stomach. The effect of various formulation variables namely the level of wicking agent (Sodium lauryl sulphate), osmotic agent in the osmotic core, the level of pore former (Guar gum) in SPM, were studied on physical parameters and drug release characteristics of developed formulations. The SEM studies showed the formation of *in-situ* delivery pores in the membrane from where the drug release occurred, and the numbers of pores formed were directly related to the initial level of pore former (Guar gum) in SPM. The results of in-vitro dissolution study and release kinetics showed the formulation F7 as the best formulation.

**Keywords:** Colon targeted; Tinidazole; semi permeable membrane; Guar gum; Microbially Activated; osmotic technology.

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## INTRODUCTION

Tinidazole is the most preferred choice of drug for intestinal amoebiasis. This drug is to be delivered to the colon for its effective action against *Entamoebe histolytica* wherein the trophozoites reside in the lumen of the caecum and large intestine and also adhere to the colonic mucus and epithelial layers. But the pharmacokinetic profile of Tinidazole indicates that the drug is completely and promptly absorbed after oral administration [1]. The administration of this drug in conventional Tablet dosage form provides minimal amount of Tinidazole for local action in the colon, still resulting in the relief of amoebiasis, but with unwanted systemic effects. Thus there is strong clinical need and market potential for a delivery system that will deliver maximum amount of tinidazole to the colon in controlled manner [2].

Colon targeted delivery systems are well recognized and documented to deliver most of the drugs to colon. In the past, various primary approaches for colon targeted delivery, such as, prodrugs approach, pH, time and pressure dependent systems, have achieved limited success. Osmotic drug delivery system (ODDS) utilizes the principle of osmotic pressure for controlled delivery of drugs [3, 4, and 5]. Drug release from these systems is independent of pH and other physiological parameter to a large extent and exhibit significant *in-vitro–in vivo* correlation. Drug delivery from ODDS follow zero-order kinetic hence provides better control over *in-vivo* performance. Various types of osmotic pumps have been reported to target the drug to colon for local or systemic therapy. These systems were essentially time dependent systems. High variation of gastric retention time makes these systems complicated in predicting the accurate location of drug release.

With all these considerations in mind, we designed microbially activated osmotic delivery systems (MAODS) for colon-targeted delivery of tinidazole. Colon targeted delivery systems of Tinidazole Tablets were developed based on osmotic technology. The developed systems consisted of osmotic core (drug, osmotic agent and wicking agent), coated with semi permeable membrane (SPM) containing Guar gum as pore former, coated core were then further coated with enteric coating to protect the system from acidic environment of stomach. The effect of various formulation variables namely the level of wicking agent (Sodium lauryl sulphate), osmotic agent (Mannitol and Fructose) in the osmotic core, the level of pore former (Guar gum) in SPM, and the thickness of SPM, were studied on drug release characteristics of developed formulations.

During its transit through the GIT, MAODS remains intact in the stomach due to the enteric-coating layer, but this layer will dissolve in the small intestine, where pH is above 6, and fluid is imbibed into the core due to osmotic pressure gradient across SPM. The continuous imbibition of core forms a saturated solution of drug within the device. When MAODS reaches the colon, guar gum (pore former) in the semi permeable membrane is specifically degraded by micro flora of the colon and thereby results in an *in situ* formation of delivery pores [6]. The saturated solution of drug is delivered from these delivery pores at a relatively constant release rate for up to 12 hr in the colon.

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## MATERIALS AND METHODS

### MATERIALS

Tinidazole was a gift sample from Spic Pharmaceutical Ltd., Chennai, India. Following chemicals and excipients were purchased from commercial sources and used as such: Eudragit S-100, Cellulose acetate, Polyvinyl pyrrolidone (PVP K-30), Microcrystalline cellulose (Avicel PH-101), Magnesium stearate, Talc, Sodium chloride, Fructose, Mannitol, PEG-400, Sodium lauryl sulphate, Guar gum were purchased from Loba Chemie Pvt.Ltd., Mumbai, India. Acetone, methanol, ethanol were purchased from Fisher Scientifics, Mumbai, India.

### METHODS

#### Differential Scanning Calorimetry (DSC)

The DSC analysis of pure drug, drug+ Mannitol, drug+ Fructose, drug+ Guar gum, drug+ SLS, drug+ Eudragit S-100 were carried out using a Shimadzu DSC 60, (Japan) to evaluate any possible drug-polymer interaction. The 2 mg sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-300ºc at heating rate of 10ºc /min under nitrogen flow of 30ml/min [7].

#### Characterization of Powder Blend

The Tablet blend were evaluated for their bulk density, tapped density, compressibility index, angle of repose and Hausner ratio. The tapping method was used to determine the bulk density, tapped density, percent compressibility index and Hausner ratio.

$$\text{Compressibility index} = [\rho_t - \rho_b / \rho_t] \times 100$$

$$\text{Hausner ratio} = \rho_t / \rho_b$$

Where $\rho_t$ = tapped density

$\rho_b$ = initial bulk density of Tablet blend.

Angle of repose θ of the Tablet blend measures the resistance to particle flow and was determined by fixed funnel method [8, 9].

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## Preparation of Core Tablets

**Table 1: Composition of Core Tablets**

| Sr.No. | Ingredients* | Formulations | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
| 1 | Tinidazole | 150 | 150 | 150 | 150 | 150 | 150 | 150 |
| 2 | Mannitol | 100 | 70 | 70 | 70 | 70 | 58 | 81 |
| 3 | Fructose | - | 80 | 80 | 80 | 80 | 66 | 93 |
| 4 | SLS | - | - | 15 | 25 | 35 | 25 | 25 |
| 5 | PVP K 30 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| 6 | MCC PH 102 | 85.8 | 35.8 | 20.8 | 10.8 | 0.8 | 36.8 | - |
| 7 | Talc | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 |
| 8 | Magnesium Stearate | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

* All quantities are in mg.

Core Tablets of Tinidazole (150 mg) were prepared by direct compression and batch size was kept as 100 Tablets. Formula of different core formulations of Tinidazole is listed in Table 1. Tinidazole was mixed with fructose and Mannitol for 10 min. After passing this mixture through #30 mesh sieve, Sodium lauryl sulphate (SLS) and Microcrystalline cellulose (MCC) were added in geometric dilution and mixing continued for additional 10 min. To this mixture Talc and Magnesium stearate each passed through #60 mesh sieve were added and mixing continued for additional 10 min. The blend was then directly compressed into Tablets having average weight of 360 mg using a sixteen station Tablet punching machine (Cadmach, Ahmadabad, India) fitted with 9 mm round flat punches.

## Preparation of Osmotic Delivery System (ODS)

**Table 2: Composition of Osmotic Delivery System (ODS):**

| Sr.No. | Ingredients | Coating Formula |
|---|---|---|
| 1 | Cellulose Acetate* | 2.00 |
| 2 | PEG 400* | 0.39 |
| 3 | Guar Gum* | 0.60 |
| 4 | Acetone# | 80 |
| 5 | Methanol# | 20 |

* All quantities are in mg;
# All quantities are in ml.

ODS were prepared by coating of core Tablets with a SPM in a conventional laboratory coating pan having outer diameter of 20 cm (Scientific Instrument, New Delhi, India) fitted with three baffles placed at angle of 120° [10]. The composition of coating solutions used for coating of Tinidazole Tablets is given in Table 2. Various components of coating solution were added to solvent mixture in sequential manner. The component added first was allowed to dissolve

before next component was added. Coating process was started on a batch of 100 Tablets; pan speed was maintained at 20 rpm and hot air inlet temperature was kept at 38-42 °C. The manual coating procedure based on intermittent spraying and coating technique was used with spray rate of 4-5 ml/min. Coat weight and thickness were controlled by the volume of coating solution consumed in coating process. In all the cases coated Tablets were dried at 50 °C for 10 h before further evaluation.

**Preparation of Microbially Activated Osmotic Delivery Systems (MAODS)**

MAODS were prepared by enteric coating of ODS with Eudragit S-100 (10% w/v in ethanol) to give enteric coating. Coating process was started on a batch of 100 SPM coated Tablets at a time and exactly same method was followed as explained above with hot air inlet temperature was kept at 40- 42 °C and spray rate of 2- 4 ml/min [11]. Coating was continued until uniform coating was obtained on the SPM coated core Tablets. In all cases coated Tablets were dried at 50 °C for 4 h before further evaluation.

**Evaluation of colon targeted tinidazole tablets**

The prepared Colon Targeted Tinidazole Tablets were evaluated for Dimension (Diameter and Thickness) using 10 Tablets (Vernier calipers), uniformity of weight using 20 Tablets (Shimadzu BL-220H analytical balance), hardness using 6 Tablets (Monsanto hardness tester), friability using 20 Tablets (Roche type friabilator).

**Drug content**

Weighed and powdered 20 Tablets. Weighed accurately a quantity of the powder containing about 0.15 g of Tinidazole, added 20 ml of methanol, shaken well and added sufficient methanol to produce 100.0 ml. Mixed well and filtered. Diluted 10 ml of the solution to 100 ml with methanol and further diluted 10 ml of this solution to 100 ml with methanol. Measured the absorbance of the resulting solution at the maximum at about 310 nm. Calculated the content of Tinidazole by taking 356 as the specific absorbance at 310 nm [12].

***In-vitro* release studies**

The developed formulations of Tinidazole were subjected to *in-vitro* drug release studies. These studies were carried out using a USP XXIV dissolution rate test apparatus (Apparatus 1, 50 rpm, 37 °C) (Campbell Electronics, Mumbai, India). The Tablets were tested for drug release for 2 h in simulated gastric fluid (SGF) (pH 1.2, 900 ml) as the average gastric emptying time is about 2 h. Then the dissolution medium was replaced with simulated intestinal fluid (SIF) (phosphate buffer pH 7.4, 900 ml) and tested for drug release for 3 h as the average small intestinal transit time is about 3 h. Then the dissolution medium was further replaced with 900 ml of simulated colonic fluid (SCF)[13] (pH 6.8 phosphate buffered saline containing $10^9$ CFU/ml of *Bacteroides fragilis*).The *Bacteroides fragilis* was collected from rat

fecus and cultured in alternate Thioglycollate medium using Resazurin as indicator [14,15]. The dissolution study was continued for another 8 h. At various time intervals, 2 ml of the dissolution sample was withdrawn and the samples were centrifuged; the supernatant filtered and the filtrate was analyzed for Tinidazole by UV Visible spectrophotometer.

**Scanning Electron Microscopy studies (SEM)**

In order to elucidate the mechanism of drug release from developed formulations, surface of coated Tablets, both before and after dissolution studies, was studied using scanning electron microscope (SEM). The samples were placed on a spherical brass stub (12 mm diameter) with a double backed adhesive tape. The Tablets (coated Tablets before dissolution studies) were mounted as such on the specimen stub. On the other hand, small sample of the coating membrane was carefully cut from the exhausted shells (after 13 h of dissolution studies) and dried at 50 °C for 6 h. The mounted samples were sputter coated for 5 to 10 min with gold using fine coat ion sputter and examined under SEM (JEOL, JSM-6100, Japan) [16].

## RESULT AND DISCUSSION

The prepared Colon Targeted Tinidazole Tablets were evaluated for thickness, weight variation, hardness, friability, drug content, *in- vitro* drug dissolution studies and stability studies. All the studies were performed in triplicate, and results are expressed as mean ± SD.

**Characterization of powder blend**

**Table 3: Flow properties of powder**

| Formulation Code | Angle of repose (θ)* | Bulk density (gm/ml)* | Tapped density (gm/ml)* | Hausner ratio (HR)* | Bulkiness* | Carr's index (IC)* |
|---|---|---|---|---|---|---|
| **F1** | 24.30±0.44 | 0.6545±0.0067 | 0.7537±0.0088 | 0.8631±0.0098 | 1.5369±0.016 | 13.6733±0.98 |
| **F2** | 27.34±0.69 | 0.6571±0.0068 | 0.7495±0.0006 | 0.8766±0.0084 | 1.5219±0.015 | 12.3286±0.84 |
| **F3** | 26.56 | 0.6434±0.0011 | 0.7717±0.0096 | 0.8338±0.0113 | 1.5541±0.002 | 16.6119±1.12 |
| **F4** | 28.11±0.43 | 0.6497±0.0061 | 0.7397±0.0088 | 0.8801±0.0129 | 1.5391±0.014 | 12.1613±0.97 |
| **F5** | 28.26±0.22 | 0.6363±0.0062 | 0.7539±0.0094 | 0.8440±0.0164 | 1.5463±0.015 | 15.6002±1.64 |
| **F6** | 26.16±0.23 | 0.6706±0.0152 | 0.7613±0.0096 | 0.8811±0.0302 | 1.4915±0.034 | 11.8887±3.02 |
| **F7** | 25.36±0.70 | 0.6476±0.0064 | 0.7674±0.0091 | 0.8440±0.0161 | 1.5407±0.013 | 15.5952±1.61 |

*All the values are expressed as mean± SE, n=3.

The powders prepared for compression of Tablets were evaluated for their flow properties, the results were shown in Table 3. Angle of repose was in the ranges between 24.30±0.44 and 28.26±0.43, indicating good flow property for all formulations. The bulk density of the powder formulation was in the range of0.6363±0.0062gm/ml and 0.6706±0.0152 gm/ml; the tapped density was in the range of 0.7397±0.0088 gm/ml and 0.7717±0.0096 gm/ml, which

indicates that the powder was not bulky. The Carr's index was found to be in the range of below 16% indicating that the powders have a good compressibility. The Hausner ratio was found to be in the range of <1.25, indicating good flow properties. These values indicate that the prepared powder blend exhibited good flow properties.

**Differential Scanning Calorimetry (DSC)**

The possible drug polymer interaction can be studied by thermal analysis. Tinidazole exhibits a sharp endothermic peak at 133.55 °C shown in Figure 1a, which corresponds to its melting point. The Tinidazole + Mannitol exhibit a sharp endothermic peak at 133.28ºC, Tinidazole + Fructose exhibit a sharp endothermic peak at 126.39ºC, Tinidazole + Guar gum exhibit a sharp endothermic peak at 130.32ºC, Tinidazole + SLS exhibit a sharp endothermic peak at 130.84ºC, Tinidazole + Eutragit exhibit a sharp endothermic peak at 131.14ºC, shown in Figure 1b, 1c, 1d, 1e and 1f respectively. Hence DSC study shows that there is no any drug polymer interaction.

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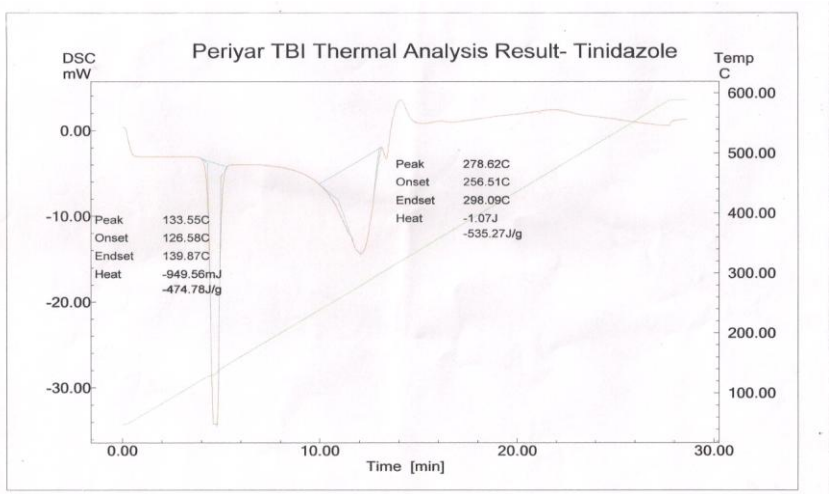


**Figure 1a: DSC thermal analysis of Tinidazole**

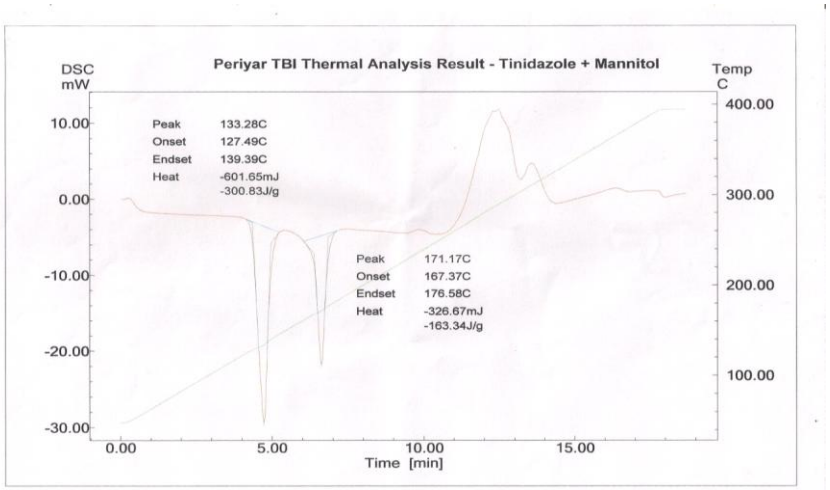


**Figure 1b: DSC thermal analysis of Tinidazole + Mannitol**

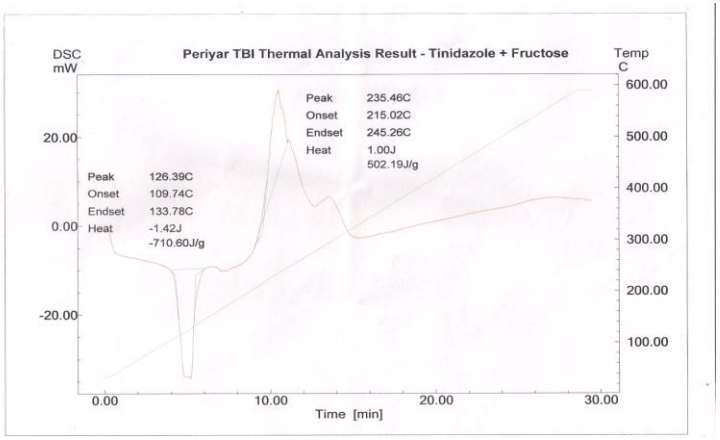


**Figure 1c: DSC thermal analysis of Tinidazole + Fructose**

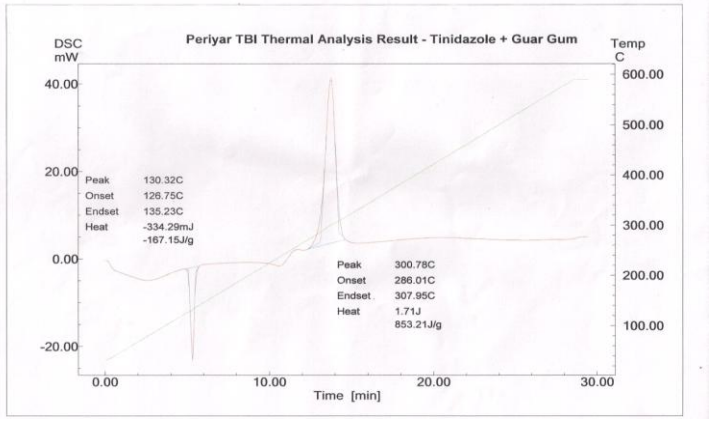


**Figure 1d: DSC thermal analysis of Tinidazole + Guar gum**

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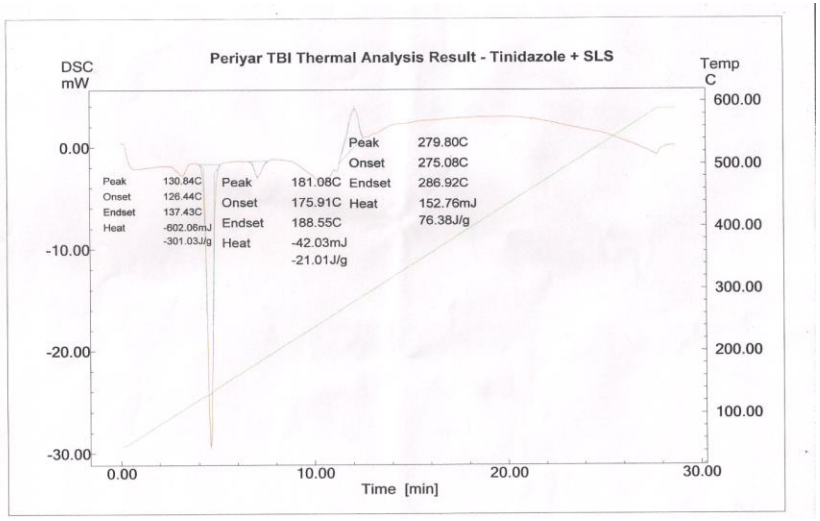


**Figure 1e: DSC thermal analysis of Tinidazole + SLS**

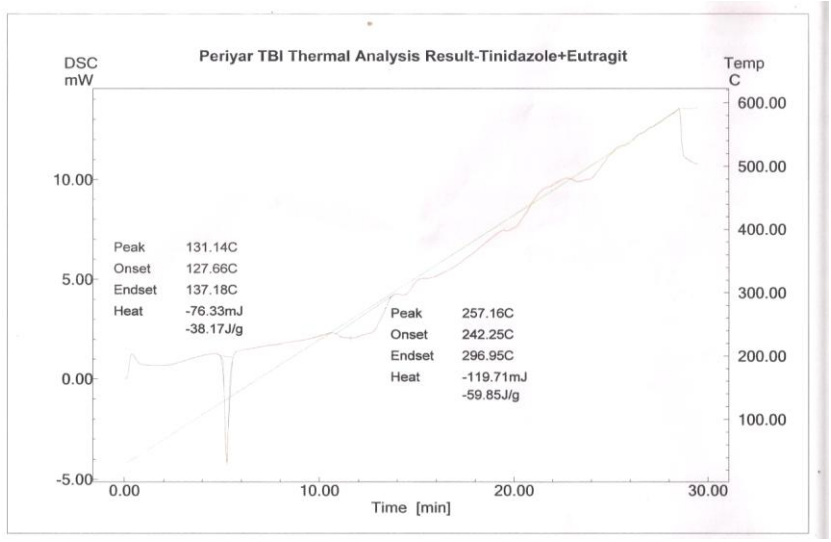


**Figure 1f: DSC thermal analysis of Tinidazole + Eutragit**

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## Physicochemical evaluation of colon targeted Tinidazole tablets

**Table 4a: Evaluation of core Tablets**

| Code | Thickness (mm)* | Weight variation test (%) | Hardness ($kg/cm^2$)# | % Friability |
|---|---|---|---|---|
| **F1** | 3.138±0.009 | 0.424±0.459 | 5.16±0.25 | 0.092 |
| **F2** | 3.141±0.011 | 0.394±0.413 | 6.16±0.25 | 0.092 |
| **F3** | 3.135±0.012 | 0.403±0.475 | 6.50±0.31 | 0.076 |
| **F4** | 3.142±0.008 | 0.351±0.460 | 6.58±0.37 | 0.092 |
| **F5** | 3.138±0.011 | 0.552±0.665 | 6.33±0.25 | 0.077 |
| **F6** | 3.141±0.009 | 0.266±0.186 | 6.66±0.25 | 0.076 |
| **F7** | 3.141±0.003 | 0.327±0.277 | 6.75±0.27 | 0.074 |

*All the values are expressed as mean± SD, n=10; # All the values are expressed as mean± SD, n=6.

**Table 4b: Evaluation of Osmotic Delivery System**

| Code | Thickness (mm)* | Weight variation test (%) | Hardness ($kg/cm^2$)# |
|---|---|---|---|
| **F1** | 3.256±0.018 | 0.137±0.070 | 6.83±0.25 |
| **F2** | 3.264±0.015 | 0.207±0.157 | 7.50±0.54 |
| **F3** | 3.258±0.017 | 0.172±0.184 | 8.25±0.27 |
| **F4** | 3.254±0.016 | 0.127±0.098 | 8.41±0.20 |
| **F5** | 3.246±0.013 | 0.127±0.176 | 7.75±0.27 |
| **F6** | 3.262±0.016 | 0.207±0.218 | 8.25±0.27 |
| **F7** | 3.254±0.009 | 0.257±0.186 | 7.75±0.52 |

*All the values are expressed as mean± SD, n=10; # All the values are expressed as mean± SD, n=6.

**Table 4c: Evaluation of Microbially Activated Osmotic Delivery System**

| Code | Thickness (mm)* | Weight variation test (%) | Hardness ($kg/cm^2$)# | Drug Content (%) # |
|---|---|---|---|---|
| **F1** | 3.362±0.014 | 0.156±0.113 | 8.16±0.25 | 99.18±0.13 |
| **F2** | 3.370±0.010 | 0.239±0.111 | 9.08±0.37 | 99.54±0.15 |
| **F3** | 3.368±0.013 | 0.217±0.081 | 9.33±0.25 | 99.56±0.05 |
| **F4** | 3.368±0.013 | 0.310±0.239 | 9.08±0.37 | 99.41±0.18 |
| **F5** | 3.368±0.010 | 0.256±0.026 | 9.00±0.44 | 99.19±0.12 |
| **F6** | 3.354±0.013 | 0.183±0.090 | 9.25±0.27 | 99.29±0.07 |
| **F7** | 3.364±0.015 | 0.171±0.163 | 9.16±0.25 | 99.64±0.04 |

*All the values are expressed as mean± SD, n=10; # All the values are expressed as mean± SD, n=6.

The Colon Targeted Tinidazole Tablets were off-white, smooth, and flat shaped in appearance. The physicochemical characterizations of core Tablets, ODS and MAODS were

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performed and the results were shown in Table 4a, 4b and 4c respectively. The thickness of Colon Targeted Tinidazole Tablets was measured by Vernier caliper and was ranged between 3.135±0.012 and 3.370±0.010mm. The weight variation for different formulations (F1 to F7) was found to be 0.127±0.098 % to 0.552±0.665 %, showing satisfactory results as per Indian Pharmacopoeia (IP) limit. The hardness of the Colon Targeted Tinidazole Tablets was measured by Monsanto tester and was controlled between 5.16±0.25 and 9.25±0.27 kg/cm2 for both uncoated and coated tablets. The friability of uncoated tablets was below 1% for all the formulations, which is an indication of good mechanical resistance of the tablet. The percentage of drug content for F1 to F7 was found to be in between 99.18±0.13 to 99.64±0.04 of Tinidazole, it complies with official specifications.

## *IN-VITRO* RELEASE STUDY

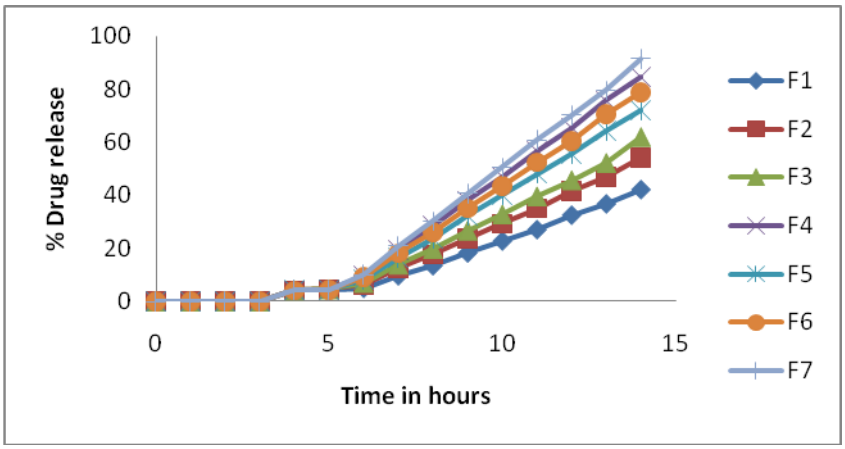


**Figure 2: *In-vitro* drug release profile of all formulation**

*In-vitro* dissolution studies of all the formulations of Colon Targeted Tinidazole Tablets were carried out in pH 1.2, pH 7.4, pH 6.8 buffer solutions. The study was performed for 14 hours, and percentage drug release was calculated at 1 hours time intervals. The results of *in-vitro* dissolution studies of all formulations were shown in Figure 2. *In-vitro* release profiles of these formulations were compared. The formulation F4 was containing 7% of SLS shows good release on comparing with formulation F3 and F5 containing SLS 4% and 10% respectively. Based on the formulation F4, the effect of osmotic agent was studied by decreasing the level of Mannitol and Fructose concentration (34%) in formulation F6 and increasing the Mannitol and Fructose concentration (46%) in formulation F7.The *in-vitro* drug release showed the formulation F7 containing 46 % w/w osmotic agent shows release of 91.57% in 14 hours. It is clearly evident that with increase in level of osmotic agent there is significant increase in drug release.

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**Kinetic modeling of drug release**

The data obtained from *in-vitro* dissolution studies were fitted in different models viz. zero order, first order, Higuchi and Korsemeyer- Peppas equation, the results were shown in Table 5. The zero order plots were found to be fairly linear as indicated by their high regression values ($r^2$ = 0.9995 to 0.9999). To confirm the exact mechanism of drug release from these tablets, the data were fitted according to zero order.

**Scanning Electron Microscopy studies (SEM)**

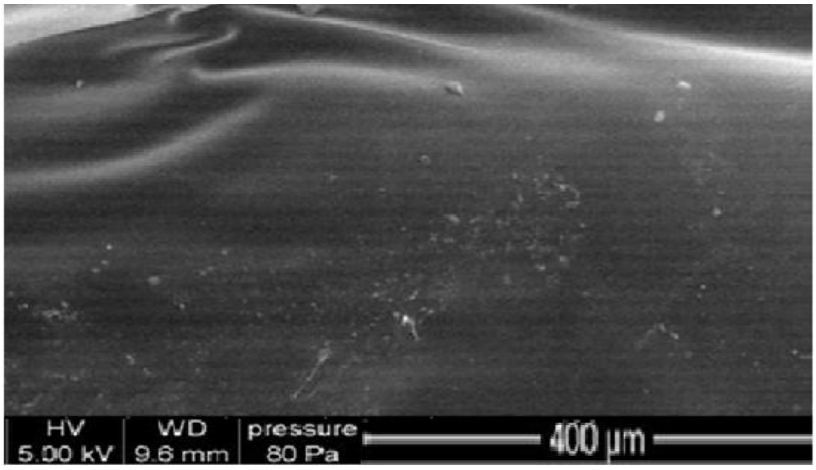


**Figure 3a: SEM photograph of Formulation F7 before in-vitro studies**

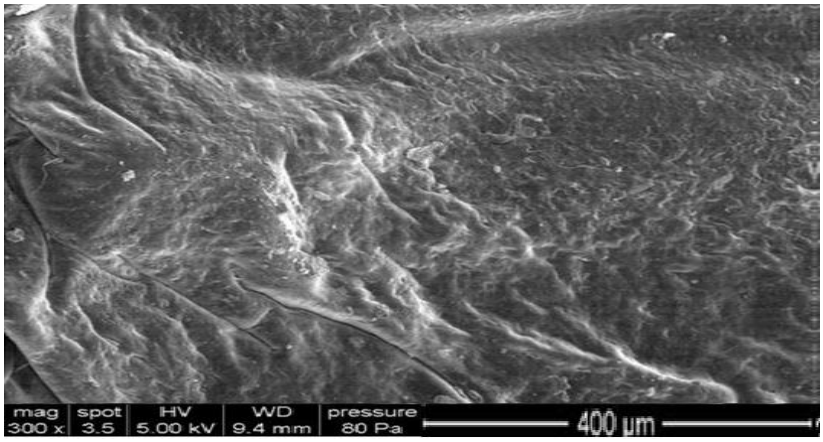


**Figure 3b: SEM photograph of Formulation F7 after in-vitro studies**

Results of SEM studies of Formulation F7 before and after in-vitro dissolution studies were shown in Figure 3a and 3b. From this it is evident that the formation of pores in the membrane after coming in contact with simulated colonic fluid and these pores will act as orifice for the release of drug in colon.

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## CONCLUSION

A microbially activated osmotic drug delivery (MAODS) for colon targeted Tinidazole Tablets was developed and evaluated for physicochemical parameters such as appearance, physical properties, drug content and *in-vitro* dissolution studies and stability studies. All the physical characteristics of the formulations like thickness, hardness, friability, drug content, and *in-vitro* dissolution study were found to be well within the limits and official standards. Based on the *in-vitro* drug release pattern the formulation F7 was concluded as best formulation among all prepared formulation for the treatment of Amoebiasis.

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## REFERENCES

[1] Samuel L Stanley. Trends in Parasitology 2001; 17 (6):280-285.
[2] Naikwade Sonali R, Kulkarni Pratibha P, Jathar Shripad R, Bajaj Amrita N. DARU 2008; 16 (3): 119-127.
[3] Ahrabi S F, Madsen G, Dyrstad K, Sande S A, Graffner C. Eur J Pharm Sci 2000; 10: 43-52.
[4] Hu Z, Shimokawa T, Ohno T, Kimura G, Mawatari S, Kamitsnna M, Yoshikawa Y, Masuda S, Takada K. J Drug Target 1999; 7: 223-232.
[5] Niwa K, Takaya T, Morimoto T, Takada I. J Drug Target 1995; 3: 83- 89.
[6] Sinha V R, Kumria R. Eur J Pharm Sci 2003; 18: 3-18.
[7] Patil S V, Kuchekar B S, Janugade B U. J Pharmacy Res 2009; 2(12): 1855-1856.
[8] Shah S H, Patel J K, Patel N V. Int J of PharmTech R 2009; 1: 823-833.
[9] Margret C R, Debjit B, Chiranjib, Jayakar B. Int J of Pharm Pharm Sci 2010; 2: 53-65.
[10] Ramakrishna N, Mishra B. Drug Dev Ind Pharm 2002; 28: 403-412.
[11] Abraham S, Srinath M S. Ind J Pharm Sci 2007; 69: 24-27.
[12] Indian Pharmacopoeia. Publications and information directorate (CSIR). New Delhi, 2007; (III): 1185.
[13] Krishnaiah Y S R, Muzib Y I, Bhaskar P, Satyanarayana V, Latha K. Drug Deliv 2003; 10: 263-268.
[14] www.aapsj.org/abstracts/AM_2008/AAPS2008-002500.PDF
[15] Kothawade Kishor B, Amrutkar Jitendra R, Gattani Surendra G, Patil Ulhas K. Int J Pharma Excip 2008; 83- 88.
[16] Rajan K Verma, Sanjay Garg. European J Pharm Biopharm 2004; 57: 513–525.