

FORMULATION DEVELOPMENT, OPTIMIZATION AND IN-VITRO RELEASE KINETIC STUDY ON COLON TARGETED TINIDAZOLE- GUAR GUM MICROCAPSULES

MANIDIPA DEBNATH, DR. Y. INDIRA MUZIB, S. ASHUTOSH KUMAR

Department of pharmaceuticals, Sri Padmavathi Mahila University, Tirupati, Pin-517502, Andhra Pradesh, India, A.K.R.G. College of pharmacy, Nallajerla, W.G. dist, A.P-534112, India. Email: ashu_mpharm2007@rediffmail.com

Received: 15 Jan 2013, Revised and Accepted: 26 Feb 2013

ABSTRACT

Objective: As part of research program to investigate the controlled and targeted delivery of drug to the colon, an attempt was made to prepare microcapsules of Tinidazole to produce local effect on colon for better control of condition like amoebiasis.

Method: Tinidazole microcapsules were fabricated through DOE using Guar gum as an encapsulating agent. Cross-linked Guar gum microspheres were prepared by Emulsion Polymerization Technique using Glutaraldehyde as a cross-linking agent.

Results: The microcapsules were characterized for % Yield (32-99%) Entrapment efficiency (22-95%w/w), Particle size (32.5- 525µm), Shape (Spherical), Surface morphology (Smooth and porous), FT-IR (No Drug-Polymer interaction). The In-Vitro dissolution studies for the selected formulations (F1, F2, F6, F14, F16, and Fopt) were carried out at different pH values without & with enzymes. The %CDR at pH 1.2 without enzymes was less than 4%, at pH 4.5 less than 8%, at pH 6.0 less than 13%, at pH 6.8 less than 16% and at pH 7.2 buffer was less than 18%. The %CDR at pH 6.8 enriched with α -Galactosidase & Galactomannase enzymes of all formulations were more than 66% within an hour. While at pH 7.2 with enzymes all formulations release 90% drug within an hour. Release kinetics of all formulations obeyed Korsmeyer-Peppas model with Super case II relaxational release mechanism ($n > 0.89$).

Conclusion: In-vitro drug release mechanism of Tinidazole-Guar gum microspheres were justifying the desire aim of the research program.

Keywords: Tinidazole, Guar Gum, Glutaraldehyde, α -Galactosidase, Galactomannase, Emulsion polymerization technique.

INTRODUCTION

Oral delivery of drugs in the colon is valuable in the treatment of diseases [1] of colon like colon cancer, diarrhea, inflammation, constipation, crohn's disease etc where by high local concentration can be achieved while minimizing side effects. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability [2]. In addition, the colon has a long retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. The approaches used in the colonic delivery of drugs include Timed release approaches, Biodegradable polysaccharides, Prodrugs, Hydrogels, Osmotically regulated, Bio-adhesive polymers etc [3]. In recent years, microencapsulation technique has gained increasing importance for oral controlled drug delivery systems [4]. The techniques like emulsion polymerization, fluidized bed or air suspension, coacervation and phase separation, spray drying and spray congealing, pan coating, solvent evaporation etc are widely using for microencapsulation [5]. In present work emulsion polymerization technique was adopted to prepare microcapsules. Several publications have described drug-containing microspheres using the Guar gum as the encapsulating materials [6, 7]. Guar gum is a natural polymer

which is chemically galactomannan polysaccharide. This polymer does not get absorbed throughout the GIT but gets degraded by the enzymes alpha galactosidase and galactomannase secreted by the gut micro flora [9]. This property of guar gum was utilized to encapsulate Tinidazole and target the release into the colon. Tinidazole is a prototype of nitroimidazole [10]. It is an anti parasitic drug used against protozoan infections [11]. It is presently marketed under the name TINDAMAX. The drug is selected for colon targeting because the colon is the major site of residence for several types of worms [12].

Experimental Design

A full 2⁴ factorial design [13] was introduced to optimize the formulation of Tinidazole loaded cross linked Guar gum microcapsules. Entrapment efficiency was considered as a measurable parameter for this study.

A design matrix comprising of 16 experimental runs was constructed using DOE Pro XL Software to investigate the effect of four factors Guar gum, Tween 80, Span 80, and Glutaraldehyde on the response variable i.e. % Entrapment efficiency. Drug quantity (4 g), volume of aqueous phase (30 ml) and oil phase (50 ml), stirring speed (2000 rpm) and temperature (70°C) were kept constant.

| Formulations | Guar gum (g) | Tween-80 (ml) | Span-80 (ml) | Glutaraldehyde (as 25%aqueous solution) (ml) |
|--------------|--------------|---------------|--------------|--|
| F1 | L | L | L | L |
| F2 | H | L | L | L |
| F3 | L | H | L | L |
| F4 | H | H | L | L |
| F5 | L | L | H | L |
| F6 | H | L | H | L |
| F7 | L | H | H | L |
| F8 | H | H | H | L |
| F9 | L | L | L | H |
| F10 | H | L | L | H |
| F11 | L | H | L | H |
| F12 | H | H | L | H |
| F13 | L | L | H | H |
| F14 | H | L | H | H |
| F15 | L | H | H | H |
| F16 | H | H | H | H |

L-Low, H-High, GG-L-1.0g, H-2.5g, Tween-80-L-0.1ml, H- 1.0ml, Span-80-L- 0.5ml, H- 2.0ml, GLT - L-2.0ml, H-4.0ml.

MATERIALS AND METHOD

Tinidazole (TN) IP was procured from Aarthi Chemicals, Guar Gum (GG) IP Glutaraldehyde (GLT) (as 25% aqueous solution), Tween 80 IP and Span 80 IP, Liquid Paraffin, Castor oil, conc. Hydrochloric Acid, conc. Sulphuric Acid, Tri Sodium Phosphate Hydrate, Sodium Acetate, Isopropyl Alcohol (IPA) were procured from S.D Fine Chemicals, Alpha Galactosidase, and Galactomannase were procured from Deer Land Enzymes U.S. All ingredients used were of AR or GR grade and were used as such without further processing. Double distilled water was used where ever required.

Preformulation studies

Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It can be defined as an investigation of Physico-chemical properties of a new drug substance alone and when combined with the excipients, to generate data useful to the formulator in developing safe, potent, bio-available and efficacious dosage form, which can be mass produced. The following parameters were selected for the Preformulation studies of the pure drug.

Identification of Pure Drug

Identification of Tinidazole was examined by FT-IR and was compared with the spectrum of Tinidazole.

Solubility Analysis

Solubility analysis was done to select suitable solvent/solvents to dissolve the drug, Polymer as well as various excipients used for the formulation of microcapsules.

Melting Point Determination

Melting point of Tinidazole was determined by using melting point apparatus (capillary tube).melting point of a drug sample is a first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by lowering as well as widening in the melting point range.

Compatibility studies

Compatibility of the TZ with GG used to formulate microcapsules was established by FT-IR. Spectral analysis of Tinidazole, Guar gum and combination of the Tinidazole with guar gum was carried out to investigate any changes in chemical composition of the drug after combining it with the excipients.

Determination of λ_{max}

10 mg of Tinidazole was dissolved in methanol and diluted to 100ml with the same solvent. 1ml of this solution was diluted to 10 ml with the methanol and examined between 220-350 nm. The maximum absorbance obtained in the graph was considered as λ_{max} . The solution has shown an absorption maximum at 300nm.

Preparation of guar gum microcapsules [8]

Guar Gum microcapsules were prepared by the Emulsion Polymerization technique. Guar gum was allowed to hydrate in 20 ml water for approximately 3 hours to achieve a viscous solution. Weighed quantity of Tinidazole was dispersed in 10 ml of water and this was added to guar gum dispersion. The above drug-gum dispersion was acidulated with 0.5 ml of concentrated sulfuric acid to give a clear viscous solution. To this Tween 80 was added under constant stirring. Separately 50 ml Castor oil was measured out in a glass beaker and Span 80 was added to it. The oil phase was placed on a hot plate fitted with a Remi stirrer. The aqueous phase was added to the oil phase in a thin stream over 2 to 3 minutes. Glutaraldehyde solution was added to the emulsion. The emulsion was stirred at 2000 rpm and heated to 75° C for 2 to 3 hours. The aqueous phase evaporated leaving microcapsules dispersed in the oil phase. The microcapsules were harvested by decantation from the oil phase and washed free from the oil and unreacted Glutaraldehyde with Iso propyl alcohol. These were then dried at 40°C for 1 hour in a hot air oven. The microcapsules were Stored in air tight containers till taken for further evaluation.

Characterization

The prepared microcapsules were characterized for the following parameters:

Particle Size [14]

Determination of average particle size of Tinidazole microcapsules was carried out by Optical Microscope(Dalal & Co, Chennai) in which stage micrometer was employed. A minute quantity of microcapsules was suspended in liquid paraffin and spreaded on a clean glass slide and average size of 600 microcapsules was determined in each batch. The values were calculated in triplicate and mean of the three readings were taken.

Surface Morphology [15]

Scanning electron microscopy has been used to determine particle distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface.SEM studies were carried out by using JEOL JSMT-330A scanning microscope (Japan).The samples of SEM were prepared by lightly sprinkling the microcapsules powder on a double adhesive tape, which was stuck on an aluminum stub. The stubs were then coated with gold to thickness of about 300A using a sputter coater. The photomicrographs were taken with the help of SEM analyzer.

Drug Entrapment Efficiency [17]

The microcapsules sample was powdered using mortar and pestle. An accurately weighed sample of 20mg of microspheres was dispersed in 47.5 ml of 0.1NHCl and 2.5ml of methanol, and sonicated for 30 minutes at a temperature of 37.5°C.The resultant solution was filtered, and the filtrate was suitably diluted with 0.1N HCl .Absorbance was measured at 300nm by using UV Spectrophotometer (E2371 Spectrophotometer)

$$\% \text{ Entrapment efficiency} = \frac{\text{Estimated Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

The entrapment efficiency values were determined in triplicate and the mean of values were calculated.

% Yield [16]

After complete drying of prepared microcapsules, weight of microcapsules was measured using digital balance. The % yield of each batch of formulation were calculated by using the formula

$$\% \text{ Yield} = \frac{\text{Total weight of Drug and Polymer}}{\text{Total weight of dried microcapsules}} \times 100$$

The values were calculated in triplicate and mean of the three readings were taken.

In-vitro drug release study (Single pH method)

The in-vitro dissolution studies have been carried out for selected formulation with entrapment efficiency more than 60%. Those formulations are as follows: F1, F2, F6, F14, F16, F opt. The different buffers used for the study were pH1.2 (2hrs), pH4.5 Acetate buffer (1hr), pH6.0- Phosphate buffer (1hr), pH6.8 Phosphate buffer (1hr), pH7.2 Phosphate buffer(1hr). For the colonic targeted study, degradation of guar gum by the bacterial enzymes is simulated by the use of alpha-galactosidase and galactomannase (enzymatic in-vitro studies) at pH 6.8(1hr) as well as at pH 7.2 buffers (1hr).

Amount of Enzymes taken

α -galactosidase-0.033u/ml =33units/lit

galactomannase-0.2u/ml =200units/lit

In-vitro drug release study (In-situ pH change method)

Dissolution study by In- situ pH change method were carried out by following way:

1. From pH 1.2 (500 ml, 2 hours) followed by pH 6.0 (745ml, 2 to 6 hours) and pH 6.8 with α -Galactosidase & Galactomannase (795ml, 6 to 14 hours).

2. From pH 1.2 (500ml, 2 hours) followed by pH 6.0 (745ml, 2 to 6 hours) and pH 7.2 with α -Galactosidase & Galactomannase (882ml, 6 to 14 hours).

The parameters for dissolution apparatus for all the above runs were kept constant as described below:

Type of apparatus: USP II (Campbell Electronics, Mumbai).

RPM: 50

Temperature: $37.5 \pm 0.5^\circ\text{C}$

N=6 samples

Mechanism of drug release and data analysis

The cumulative amount of Tinidazole release from the formulated microcapsules at different time intervals by In-situ pH change methods [(a). pH 1.2, 6 and 6.8 with α -Galactosidase and

Galactomannase, (b). pH 1.2, 6 and 7.2 with α -Galactosidase and Galactomannase] were fitted with different mathematical models such as zero order release (cumulative % drug release versus time), first order release (log cumulative of % drug remaining versus time), Higuchi model (cumulative % drug release versus square root of time) Korsmeyer-Peppas model (log cumulative % drug release versus log time) and Hixson-Crowell release (cube root of % drug remaining in matrix versus time) to characterize the mechanism of drug release.

RESULT & DISCUSSION

Identification of Pure Drug and Drug-Polymer Compatibility

Identification of TZ and TZ- GG compatibility studs were carried out by FT-IR. The obtained spectra have shown in Fig1&2. From spectra of TZ alone has compared with standard one and TZ has identified. The TZ-GG spectra has confirmed about no Drug-Polymer interaction.

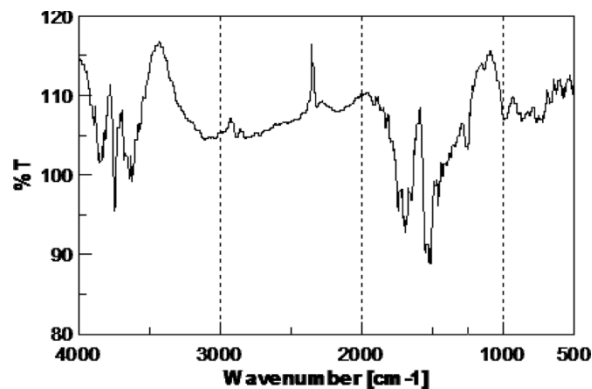


Fig. 1: It shows FT-IR Spectra of Tinidazole Guar Gum mixture

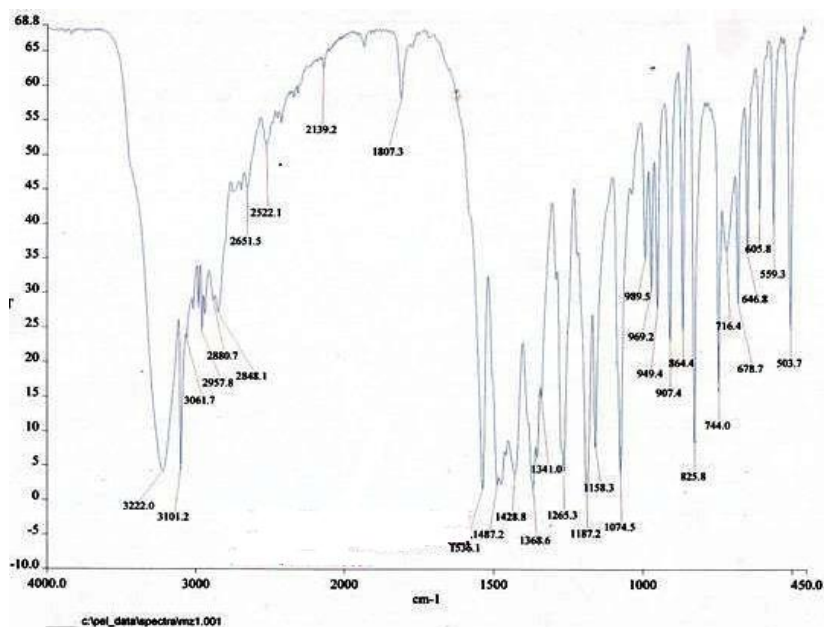


Fig. 2: It shows FT-IR Spectra of Tinidazole

Solubility Analysis

The solubility of drug as well as polymer was carried out in different solvents system. The studies revealed that polymer was freely soluble in water with swelling property. Drug was insoluble in water but was showing free solubility in diluted acids (0.01N H_2SO_4 and HCl).

Melting Point Determination

Melting point of Tinidazole was determined by using melting point apparatus (capillary tube). Melting point of a drug sample is a first

indication of purity of the sample. The presence of relatively small amount of impurity can be detected by lowering as well as widening in the melting point range. Melting point of TZ was found to be 127.5°C , which is the melting point of pure Tinidazole according to the monograph.

Determination of λ_{max}

To determine the λ_{max} of Tinidazole, absorbance was measured in between 220-350 nm. The maximum absorbance obtained in the graph was considered as λ_{max} . λ_{max} of Tinidazole was found to be 300nm.

Particle size

The particle sizes of all 16 formulations were carried out by Optical Microscopic method using Stage and Eye-piece micrometer. 600 particles from each formulation were counted. The average diameter of 600 particles was calculated by using standard formula. The average particle sizes ranged in between 32.5-525 μm . All 16 formulations values are shown in Table 2.

Entrapment efficiency [17]

Entrapment Efficiency (EE) was determined by the method previously described. The % Entrapment efficiency was varied from 22.1-94.5%w/w (Table 2). The Entrapment efficiency of Fo_{pt} [17] was calculated 98%w/w.

Surface Morphology

SEM photographs are shown in shown Fig 3. The SEM images indicate spherical shape blank microcapsules with very smooth surfaces. SEM images of Tinidazole-Guar gum microcapsules are also spherical in shape but the surfaces are rough and porous in nature.

In-vitro dissolution profile (Single pH Method):

The drug release in acid media was less than 5% at the end of 2 hours for all formulations indicating excellent protection of the drug in the stomach environment (Fig 4A). The drug release in pH 4.5 was

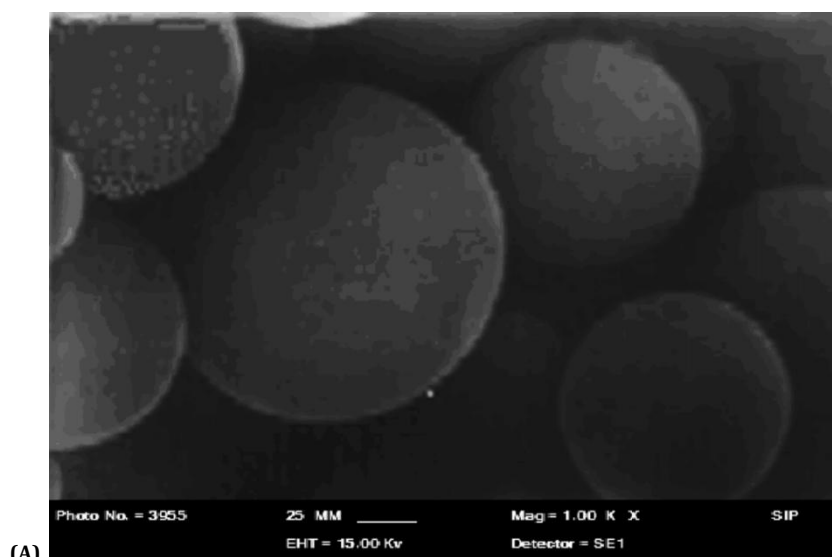
less than 10% for all formulations indicating excellent protection of the drug in the area of duodenum and upper intestine (Fig 4B). The drug release at pH 6.0, pH 6.8 and pH 7.2 which simulate the Small intestine, Large intestine and Colon was less than 20% (Fig 4C,4D,4E) This indicates that the drug release was not dependent on the pH of the gastro intestinal tract.

Further in vitro dissolution studies were conducted by adding a mixture of α -Galactosidase and Galactomannase at pH 6.8 and pH 7.2 buffers in order to mimic enzyme expression by microflora in the Large intestine and the Colon. There is significant drug release observed in enzyme enriched pH 6.8 buffer (Fig 5A). Drug release was significantly lower in formulations F1, F2 and F6 as compared to F14, F16 and F opt. This may be due to lower GLT levels in the 3 formulations leading to lesser cross linking of the Guar gum. Dissolution in this media was dependent on the GLT concentration used, indicating that when cross linkage of Guar gum was less (lower GLT) the swelling property of Guar gum was affecting the drug release. Hence the swelling properties of GG may be greater for these 3 formulations giving rise to delaying the drug release in pH 6.8. In enzyme enriched pH 7.2 buffer media, all formulations are giving drug release > 85% in 1 hour (Fig 5B). All formulations were significantly similar both in rate as well as extent. This indicated that in the lower colonic region, drug release was independent of the composition of the microcapsules and complete release of the drug was achieved.

Table 2: It shows % Yield, Particle size and Entrapment efficiency of Tinidazole-Guar gum Microspheres

| Formulations | % Yield (w/w) ^a | Particle size(μm) ^a | Entrapment efficiency(%w/w) ^a |
|------------------|----------------------------|---|--|
| F1 | 42 | 65 | 89.6 |
| F2 | 69 | 350 | 94.6 |
| F3 | 93 | 525 | 34 |
| F4 | 38.2 | 65 | 51.1 |
| F5 | 84.7 | 350 | 44.31 |
| F6 | 91 | 515 | 62.45 |
| F7 | 63 | 65 | 22.1 |
| F8 | 51 | 32.5 | 26.09 |
| F9 | 32 | 65 | 55 |
| F10 | 86 | 65 | 57.96 |
| F11 | 94.3 | 525 | 45.60 |
| F12 | 61 | 350 | 55.12 |
| F13 | 96.6 | 525 | 33.5 |
| F14 | 93.3 | 350 | 89.3 |
| F15 | 90.6 | 525 | 57.6 |
| F16 | 82 | 345 | 60.9 |
| F _{opt} | 99 | 500 | 98 |

^a Data are expressed as mean \pm SD (standard deviation), $n=3$.



(A)

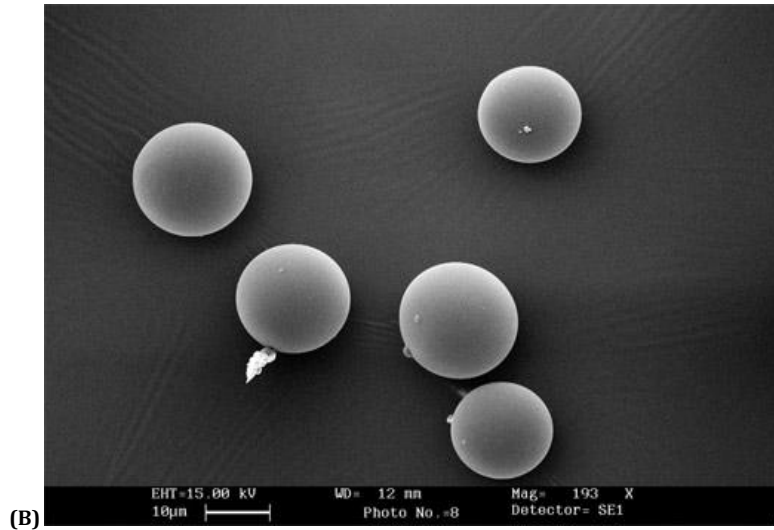
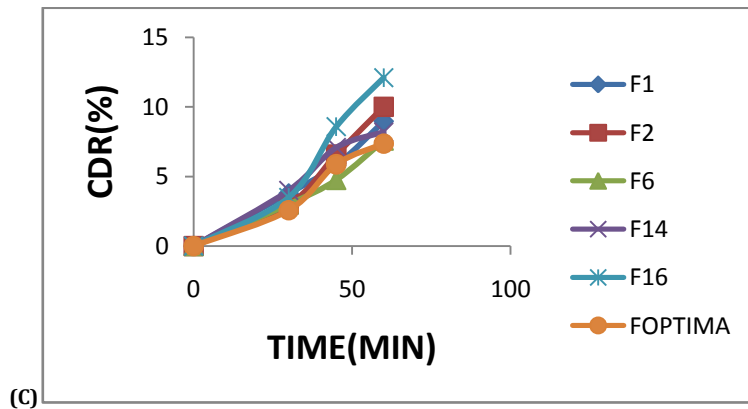
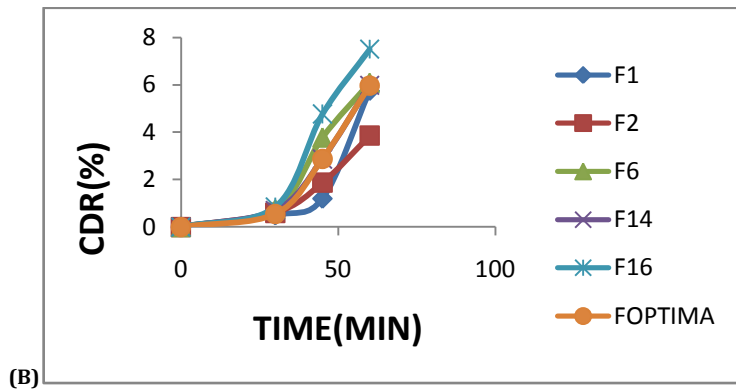
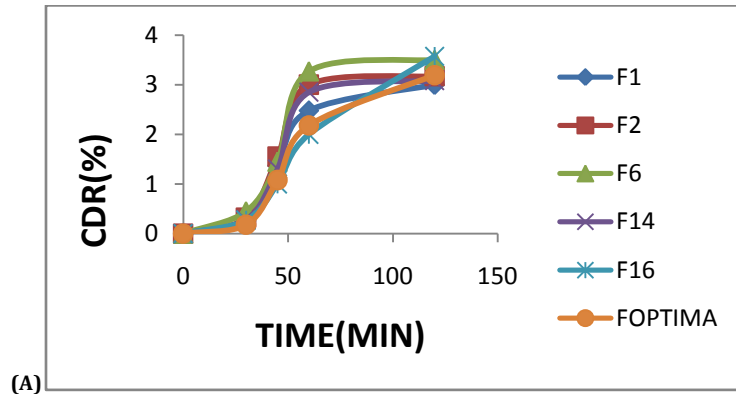


Fig. 3: It shows SEM image of TZ-GG Microcapsules (A) Blank Microcapsules (B)



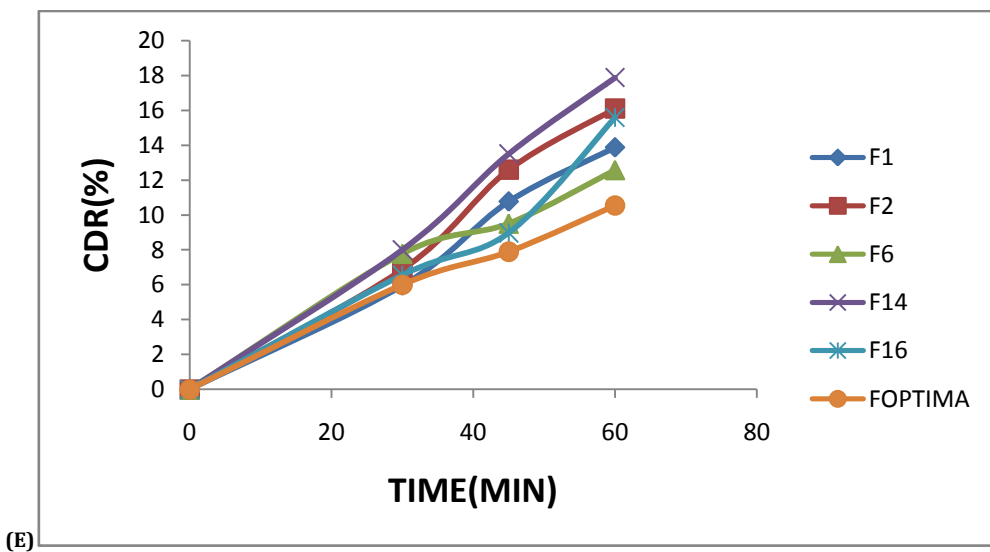
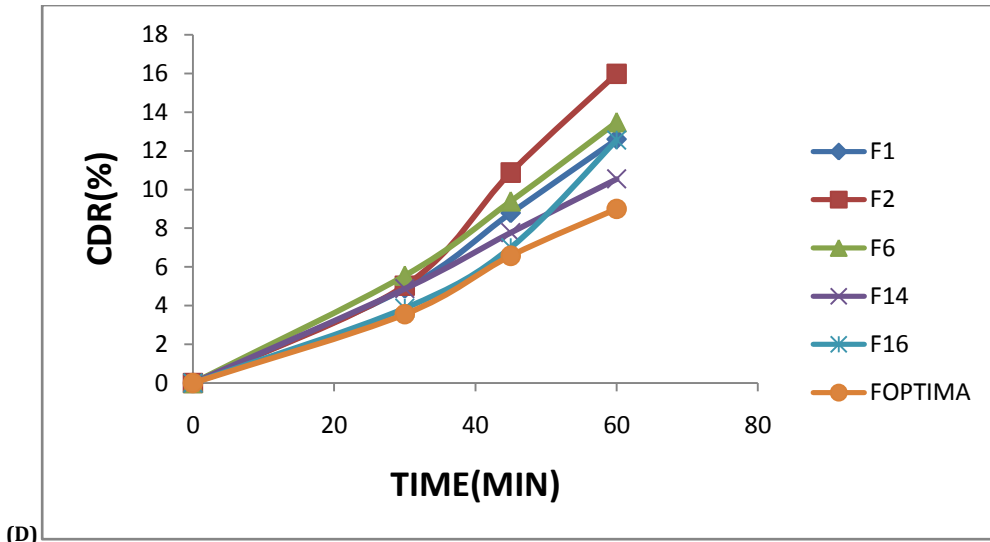
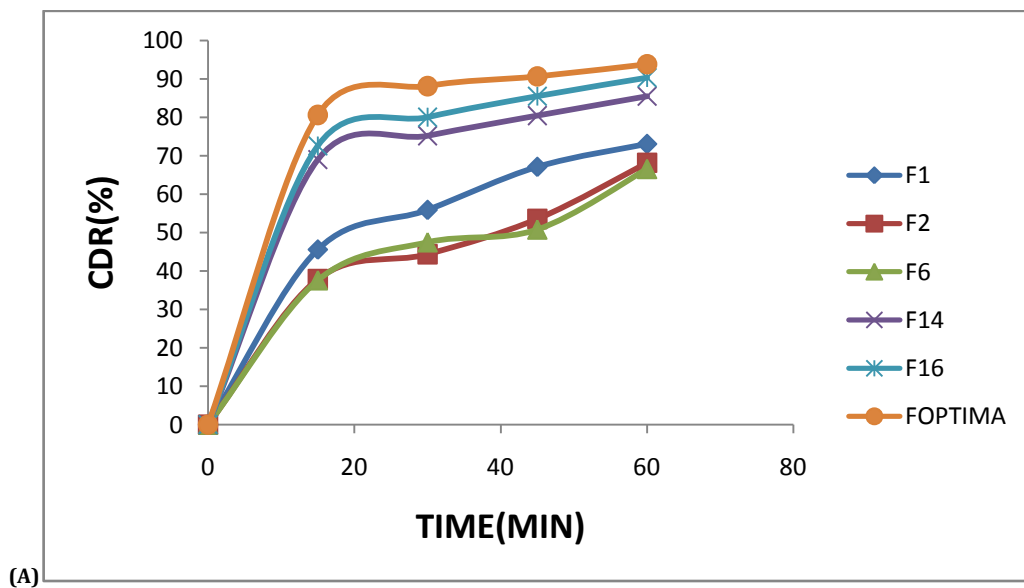


Fig. 4: It shows dissolution profile of Tinidazole-Guar gum microspheres at pH 1.2(A), pH, 4.5(B), pH 6(C), pH 6.8(D) and pH 7.2(E)



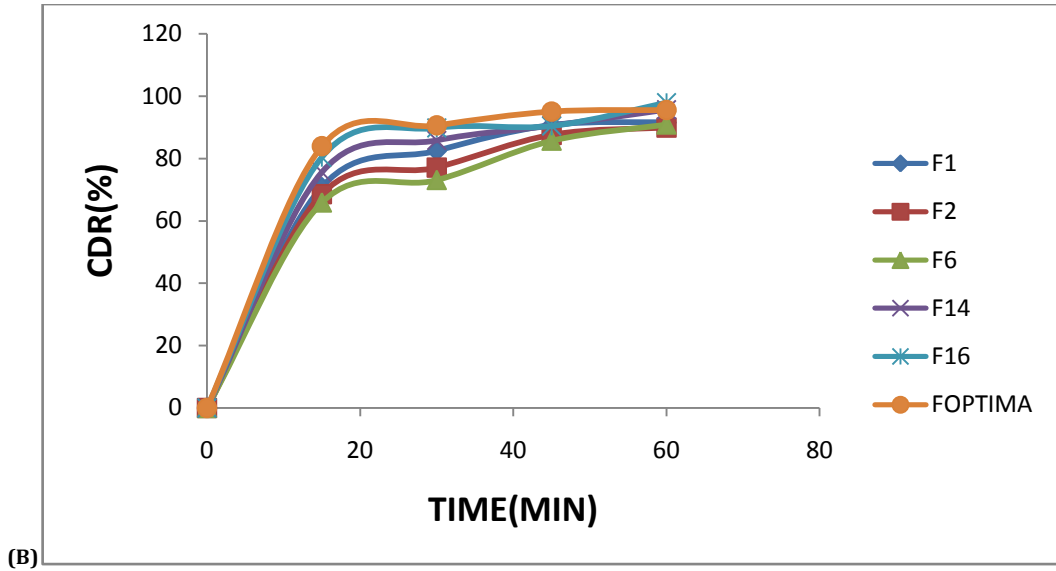


Fig. 5: It shows dissolution profile at pH 6.8(A) and pH 7.2(B) with α -Galactosidase and Galactomannase.

In-situ pH change method

To mimic the in-vitro transit time and pH changes at GI fluids, dissolution studies were carried by In-situ pH change method. The drug release patterns by In-situ pH change method were similar to those achieved by Single pH Method (Fig 6A, 6B).

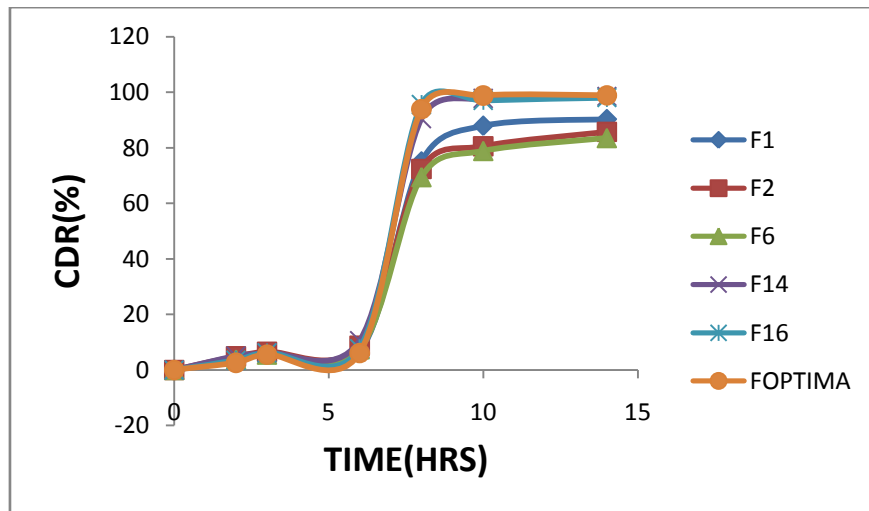


Fig. 6A: It shows disso profile by in- situ pH change method (at pH 1.2, 6.0, 6.8) with enzymes.

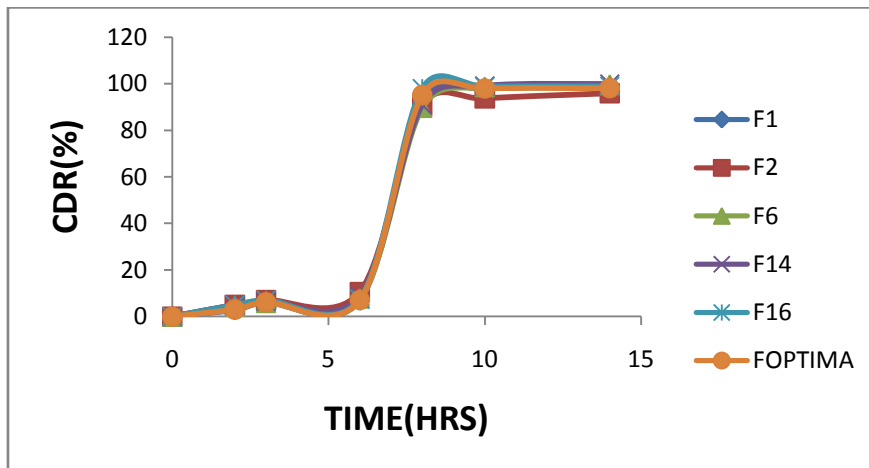


Fig. 6B: It shows disso profile by in- situ pH change method (at pH 1.2, 6.0, 7.2) with enzymes.

Mechanism of drug release and release kinetics

Drug release kinetics was fitted with different mathematical models such as zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell release. This demonstrates the pattern of the drug release. The values of Correlation coefficient indicated, all formulations exhibit release kinetics as defined by Korsmeyer-Peppas mechanism indicating that release rate was governed by

combine effects of Diffusion, Swelling and Erosion of the polymer matrix (Table 3A, 3B).

Exponential coefficient (n) value indicates the exact drug release mechanism by Korsmeyer-Peppas model. n values are depicted in (Table 4). n values at both medium revealed that all formulations followed Super case II relaxational release mechanism (n > 0.89).

Table 3A: It shows correlation coefficient values of Tinidazole-Guar gum microspheres at pH 1.2, 6, 6.8 with Enzymes

| Formulation | Zero order | Higuchi | Peppas | First order | Hixson-Crowell |
|-------------|------------|---------|--------|-------------|----------------|
| F1 | 0.817 | 0.685 | 0.887 | 0.848 | 0.852 |
| F2 | 0.829 | 0.703 | 0.882 | 0.860 | 0.849 |
| F6 | 0.827 | 0.697 | 0.903 | 0.856 | 0.846 |
| F14 | 0.803 | 0.688 | 0.914 | 0.849 | 0.833 |
| F16 | 0.776 | 0.665 | 0.892 | 0.814 | 0.791 |
| Fopt | 0.777 | 0.660 | 0.873 | 0.820 | 0.805 |

Table 3B: It shows correlation coefficient values of Tinidazole-Guar gum microspheres at pH 1.2, 6, 7.2 with enzymes

| Formulation order | Zero order | Higuchi | Peppas | First | Hixson-Crowell |
|-------------------|------------|---------|--------|-------|----------------|
| F1 | 0.781 | 0.665 | 0.885 | 0.838 | 0.812 |
| F2 | 0.798 | 0.687 | 0.914 | 0.837 | 0.817 |
| F6 | 0.799 | 0.677 | 0.891 | 0.866 | 0.852 |
| F14 | 0.798 | 0.681 | 0.898 | 0.864 | 0.851 |
| F16 | 0.769 | 0.663 | 0.875 | 0.789 | 0.774 |
| Fopt | 0.775 | 0.663 | 0.886 | 0.807 | 0.790 |

Table 4: It shows exponential coefficient (n) values of Tinidazole-Guar gum Microspheres

| Formulations | pH 1.2,6, 6.8 | pH 1.2,6, 7.2 |
|--------------|---------------|---------------|
| F1 | 1.805 | 1.89 |
| F2 | 1.695 | 1.80 |
| F6 | 1.762 | 1.834 |
| F14 | 1.822 | 1.817 |
| F16 | 1.863 | 1.805 |
| Fopt | 1.909 | 1.879 |

CONCLUSION

Colon targeting of Tinidazole can be achieved by encapsulating the drug in GG polymer. The in- vitro dissolution studies were performed individually in pH 1.2, pH 4.5, pH 6, pH 6.8 and pH 7.2 buffers and in all these media drug release was less than 20%, indicating drug release was not pH driven. Dissolution studies were also conducted in pH 6.8 and pH 7.2 media enriched with Alpha Galactosidase and Galactomannase enzymes. In pH 6.8 with enzymes, the drug release was governed by swelling effect of Guar gum, while in pH 7.2 with enzymes the drug release was independent on swelling effect of Guar gum and more than 85% release completed within an hour. Release kinetics of all formulations showed Korsmeyer-Peppas mechanism of drug release. Exponential coefficient values (n > 0.89) of the formulations proved Super case II relaxational release mechanism of drug release.

REFERENCES

- Toratora Grabowski. Principles of anatomy and physiology. 10th ed. New York: John Wiley & Sons; 2002.
- Tripathi KD. Essentials of Medical Pharmacology. 6th ed. New Delhi: Jaypee Brothers Medical Publishers; 2008.
- D.M.Brahmankar and Sunil B. Jaiswal. Biopharmaceutics and Pharmacokinetics-A Treatise. 2nd ed. Delhi: Vallabh Prakashan; 2009.
- Vyas SP, Khar RK. Targeted and controlled drug delivery-novel carrier systems. 2nd ed. New Delhi: CBS Publishers; 2002.
- Saraswathi R, Simi SP, Sankar C, Krishnan PN, Dilip C, Ameena K. Guar gum based microcapsules for colonic delivery of Albendazole: Development and In-vitro evaluation Res. J. Pharm. bio and Chem 2010; 1(4): 373.
- Rana Mazumder, Lila k Nath1, Anwarul Haque, Tarasankar Maity, Prasanta K Choudhury Formulation and in vitro

- evaluation of natural polymers based microspheres for colonic drug delivery. Int.J.pharm. Sci 2010; 2(1): 231-233.
- Ravi V, Kumar PM, Siddharamaiah. Novel colon targeting drug delivery system using natural polymers. Int. J. Pharm. Sci 2008; 70(1):108-111.
- Shukla Raj Kumar, Trivedi Piyush, Ramteke Suman, and Tiwari Akanksha. Preparation and characterization of cross-linked guar gum microspheres: Optimization using factorial design Chem. pharm. Bull 2011; 59 (2) 185-190.
- Bayliss, C.E., Houston, A.P. Degradation of guar gum by faecal bacteria. Appl. Environ. Micro. 1986; 48:626-632.
- Weissman S, Salata R, "Amoebiasis," Nelson Textbook of Pediatrics. 16th ed. Philadelphia: W.B. Saunders Company; 2000.
- United States Pharmacopoeia. Vol 1: pg.no1296. 2007
- Cada DJ, Levine T, Baker DE. Tinidazole. Formulary Drug Reviews 2004; 39(10):976-87.
- DOE Approach available on [www.google/doi/tutorial/illustration/types of doe designs](http://www.google/doi/tutorial/illustration/types%20of%20doe%20designs).
- Nasra MA, EL-Massik MA, Naggar VF, Development of Metronidazole colon specific delivery systems. Asian. J. Pharm. Sci 2007; 2(1)18-28.
- Dinesh C, Yana YK, Jaiswal D, Ghosh N, Singh HP, Mishra A. Formulation and evaluation of Satranidazole microspheres for colon targeted drug delivery. J. Pharm. Res 2009; 2(7): 1230-3.
- Jain SK, Rai G, Saraf D K, Agarwal GP. The preparation and evaluation of Albendazole microspheres for colonic delivery. Pharma. technol 2004; 67: 66-71.
- Vinay Rao, S. Manidipa, S. Ashutosh Kumar. Design space determination for preparation of colon targeted Microcapsules of Tinidazole using emulsion polymerization Technique. Pharmanest Int.J.Adv.Pharm.Sci 2012; 3(2): 103-108.