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Fabrication and Evaluation of Tinidazole Microbeads for Colon Targeting

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ABSTRACT

Objective: The purpose of present investigation was to develop and evaluate multiparticulate system exploiting pH-sensitive property and specific biodegradability of calcium alginate microbeads, for colon- targeted delivery of Tinidazole for the treatment of amoebic colitis. **Methods:** Calcium alginate beads containing Tinidazole were prepared by ionotropic gelation technique followed by coating with Eudragit S100 using solvent evaporation method to obtain pH sensitive microbeads. Various formulation parameters were optimized which included concentration of sodium alginate (2% w/v), curing time (20 min) and concentration of pectin (1% w/v). All the formulations were evaluated for surface morphology, particle size analysis, entrapment efficiency and in-vitro drug release in conditions simulating colonic fluid in the presence of rat caecal (2% w/v) content. **Results:** The average size of beads of optimized formulation (FT4) was found to be 998.73±5.12 μm with entrapment efficiency of 87.28±2.19 %. The invitro release of Eudragit S100 coated beads in presence of rat caecal content was found to be 70.73%±1.91% in 24 hours. Data of in-vitro release was fitted into Higuchi kinetics and Korsmeyer Peppas equation to explain release profile. The optimized formulation (FT4) showed zero order release. **Conclusions:** It is concluded that calcium alginate microbeads are the potential system for colon delivery of Tinidazole for chemotherapy of amoebic infection.

1. Introduction

The oral route has been the major route for drug delivery for chronic treatment of many diseases. Colon drug delivery has number of important implications in the field of pharmacotherapy. Targeting of drugs to the colon is advantageous in the treatment of diseases like amoebiasis, Crohn's disease, ulcerative colitis and colorectal cancer. To achieve successful colonic delivery, the drug needs to be protected from absorption in the upper gastrointestinal tract (GIT) and then released into the proximal colon, for targeted delivery of drugs.[1,2] Various strategies currently available to target the release of drugs to colon, include, formation of prodrug, coating of pH sensitive polymers, use of colon-specific biodegradable polymers, timed release systems, osmotic systems, and pressure controlled drug delivery systems. Among the different approaches to achieve targeted drug release to the colon, the use of polymers, especially the one which are degraded by colonic bacteria, hold great promise. Polysaccharides, which are substrates for colonic bacterial enzymes, can be exploited in colon targeting of drugs.[3-6]

Pectin is a predominately linear polymer of mainly α-(1-4)-linked D-galacturonic acid residues interrupted by 1, 2-linked L-rhamnase residues. Pectin is a polysaccharide found in the cell wall of plants. It is totally degraded by colonic bacteria but is not digested in the upper GIT. One disadvantage of pectin is its solubility. This drawback can however be adjusted by changing its degree of methoxylation, or by preparing calcium pectinate.[7]

The advantages of multiparticulate dosage forms over single

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unit preparations are uniform dispersion in the GIT, uniform drug absorption, less inter and intra individual variability and a flexible formulation process. The interest in multiparticulate as oral drug delivery systems has been growing steadily. Small particle size of multiparticulate ensures its easy passage through the upper GIT and can reach the colon quickly and be retained longer in the ascending colon.[6] Tinidazole is the drug of choice for acute and chronic amoebiasis and other protozoal disease. When taken orally as a conventional tablet, it causes gastric distress. It is distributed into virtually all tissues and body fluid. Approximately 20-25% is excreted in unchanged form from the urine and 12% is excreted in the faeces.

The objective of the present study was to develop multiparticulate delivery system for site specific delivery of Tinidazole using natural polysaccharide (pectin) and pH sensitive polymer (Eudragit S100). This system was designed so as to protect the degradation of drug in the upper part of GIT by using Eudragit S100 and to deliver Tinidazole specifically to the colon, to reduce systemic dosing, and also to alleviate gastric distress.[8,9]

2. Materials and Methods

2.1. Materials

Tinidazole was received as a gift from Aldoc Pharmaceuticals Pvt. Ltd (Kota, India). Sodium alginate and Pectin were received as gift samples from Himedia Pvt. Ltd (Mumbai, India). Calcium chloride was purchased from S. D. Fine Chem Ltd (Mumbai, India). All other solvents and reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of Microbeads

Microbeads of Tinidazole were prepared using ionotropic gelation technique. Two different sets of microbeads were prepared using sodium alginate alone and in combination with pectin. Various formulation parameters like sodium alginate concentration, curing time and pectin concentration were optimized on the basis of average diameter, entrapment efficiency and drug loading (Table 1).[10]

2.2.2. Preparation of Calcium Alginate-Pectinate Microbeads

Accurately weighed 300 mg of Tinidazole was added to 5 ml aqueous solution of sodium alginate (2% w/v) and 5ml of varying concentrations i.e. 0.3% (FT2), 0.5% (FT3)and 1.0 % w/v (FT4) of pectin in 25ml aqueous solution of calcium chloride (2 % w/v) through hypodermic syringe with needle size no. 24G. The formed beads were kept under stirring in the medium at 500 rpm for 20 min and then separated by filtration. The separated beads were washed with distilled water and vacuum dried.[4,6,11,12,13]

2.2.3. Coating of calcium alginate-pectinate microbeads

The coating of optimized calcium alginate bead formulation (FT4) was carried out with Eudragit S100 using solvent evaporation method. Beads were transferred into various concentration solutions of Eudragit S100 in acetone to obtain 5%, 10%, and 15% weight gain. The solvent was evaporated in a rotary evaporator by applying vacuum at 300 mm Hg and 50 rpm. The beads were further vacuum dried in a desiccator for 12 hours to ensure evaporation of residual solvent, if any.[6,11]

2.2.4. Characterization of Tinidazole Loaded Microbeads

2.2.4.1. Shape and Surface Morphology

Shape and surface morphology of calcium alginate microbeads was studied by Scanning Electron Microscope (SEM) (Philips 505, PW 6765/00) using gold sputter technique. The samples were prepared by lightly sprinkling the powder on a double-sided adhesive tape stuck to a stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The photomicrographs were taken within a range of 50-500 magnification.[1,5,6,11,14]

2.2.4.2. Particle size

An optical microscope fitted with digital camera (Yoko CCD 215 F, 560191, Taiwan,) was used and particle size analysis of prepared microbeads was performed by determining the diameter of randomly selected 100 microbeads using the Medical Pro software (version 3).[15]

2.2.4.3. Entrapment efficiency

Accurately weighed 100 mg of microbeads was suspended in 100 ml of methanol, vortexed for 5 min and filtered through whatman filter paper no.4. The samples were suitably diluted and analyzed spectrophotometrically at 305 nm using UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu). The drug entrapment efficiency was determined by the following relationship.[6,8,9,14,16,17]

$$\%\text{Drug entrapment efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

2.2.4.4. In vitro drug release studies in simulated gastric fluid

Eudragit coated and uncoated calcium alginate microbeads were evaluated for in-vitro drug release in simulated gastric fluid (SGF) in a USP Dissolution Test Apparatus II (Type II, Veego DA, 6 DR Japan) using 900 ml of dissolution medium, at 100 rpm and 37±0.5 ℃ maintaining sink condition throughout the study. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The dissolution medium was maintained at pH 1.2 for the first 2 hours, followed by pH 7.4 for the next 3 hours. The medium was replaced by Phosphate Buffer Saline (PBS) pH 6.8 and the release was observed up to 8 hours. Samples were withdrawn at predetermined time intervals using a pipette, the tip of which was covered with filter paper to avoid drug particles. The amount of Tinidazole released was determined spectrophotometrically at 305 nm using UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan).[6,12,13,18,19]

2.2.4.5. Preparation of rat caecal contents

The experiment was performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by Institutional Animal Ethical Committee (IAEC). Rat caecal content was prepared by the method as described by Van Den Mooter et al (1994). Two Wistar albino rats of uniform body weight (150-200 g) with no prior drug treatment were maintained on normal diet and were administered 1.0 ml of 1% dispersion of pectin in water for 7 days to initiate microbial enzyme induction. Thirty minutes prior to the study, each rat was humanely killed (euthanasia) and the abdomen cut open. The caecum was isolated, ligated at both the ends, cut loose and immediately transferred into simulated colonic fluid of pH 6.8 previously bubbled with carbon dioxide. The caecal contents were individually weighed, pooled, and suspended in buffer to produce a final caecal concentration of 2% w/v.

2.2.4.6. In vitro drug release study in the presence of rat caecal contents

Eudragit coated and uncoated calcium alginate microbeads were evaluated for in vitro drug release in rat caecal content in a USP Dissolution Test Apparatus (Type II, Veego DA, 6 DR Japan) using 900 ml of dissolution medium, at 100 rpm and 37± 0.5 ℃ maintaining sink condition throughout the study.

Drug release rate studies were performed initially in GI fluid for 5 hours. From the sixth hour onwards the release study was performed in simulated colonic fluid containing rat caecal content. The experiment was performed with a continuous supply of carbon dioxide into the dissolution media to simulate the colonic environment.[6,9,12,13]

2.2.4.7. Mechanism of drug release

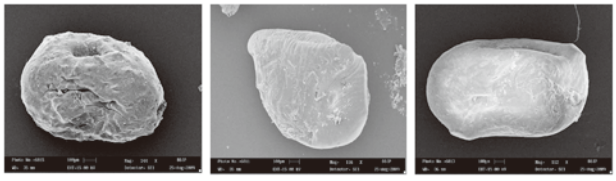
The mechanism of drug release was determined by fitting the release data into various kinetic models such as zero-order (% drug release vs time), first-order (log % drug retained vs time), Higuchi (% drug release vs square root of time) and Korsmeyer-Peppas.[20]

3. Results

Various formulation parameters like sodium alginate concentration, curing time and pectin concentration were optimized on the basis of average diameter, entrapment efficiency and drug loading. It was observed that 2% w/v sodium alginate, 1% w/v pectin in water, curing time of 20 minutes, resulted in microbeads having a spherical shape and optimum size with high entrapment efficiency as well as high drug loading (Table 2).

3.1. Surface morphology

The SEM images showed that Eudragit coated microbeads possessed smooth surface compared to uncoated beads as shown in Figure 1. The calcium alginate-pectinate microbeads (FT4), was found to have rough surface. Moreover, the dispersion of Tinidazole as fine crystalline particles was also observed on the surface of microbeads. Formulation FT1 showed faster in vitro release as compared to FT4.



a b c

Table 1

Optimization of Various Formulation Parameters*

Variables	Values	Formulation code	Average Diameter (μm)	Drug Entrapment Efficiency (%)	Drug loading (%)
Sodium alginate (%w/v)	0.5	BS1	875.21±6.22	69.40±1.75	25.11±1.18
	1	BS2	954.45±8.54	74.91±3.69	27.64±1.13
	2#	BS3	995.19±5.62	80.10±3.87	28.79±1.77
Pectin (%w/v)	0.3	BP1	903.32±7.75	83.31±3.68	26.53±2.65
	0.5	BP2	964.46±6.98	85.16±3.47	27.24±1.52
	1#	BP3	998.73±5.21	87.28±2.19	29.56±1.34
Curing time (min)	10	BC1	998.21±6.22	72.40±1.75	24.10±1.18
	20#	BC2	995.45±8.54	79.91±3.69	28.64±1.53
	40	BC3	990.19±5.62	77.10±3.87	27.79±1.77

*Mean±S. D., n=3, #optimized values

Table 2

Characterization of Various Formulations*

Formulation code	Average Diameter (μm)	Drug Entrapment Efficiency (%)	Drug Loading (%)	In-vitro (%) release after 5 hours	Shape and Appearance
FT1	995.19±5.62	80.10±3.87	28.79±1.77	97.61±3.06	Not Spherical
FT2	903.32±7.75	83.31±3.68	26.53±2.65	84.21±2.83	Spherical shape with irregular surface
FT3	964.46±6.98	85.16±3.47	27.24±1.52	80.51±1.56	Spherical shape with smooth surface
FT4	998.73±5.21	87.28±2.19	29.56±1.34	78.89±1.37	Spherical shape with smooth surface

*Mean±S. D., n=3

Fig. 1: Scanning electron micrograph (SEM) of microbeads (a) Core calcium alginate bead, (b) Core calcium alginate pectinate and (c) Coated calcium alginate-pectinate bead.

3.2. Drug Entrapment Efficiency and Drug loading

The entrapment efficiency of optimized formulation FT4 (calcium alginate-pectinate) exhibited highest drug loading and percentage entrapment efficiency values of 29.56±1.34 % and 87.28±2.19% respectively, whereas FT1 microbeads (calcium alginate) showed the least value of drug loading and percentage entrapment efficiency as 24.12±2.86 % and 71.45 ±2.36 % respectively. This may be attributed to the possible leakage of drug from calcium alginate microbeads having cracks.

3.3. Optimization of Coating Thickness

The coating thickness was optimized in terms of total weight gain (TWG) of the beads after coating with Eudragit S100 dispersion. The effect of coating thickness on in vitro drug release in simulated GIT fluids was studied. The drug release for the optimized formulation (FT4) was found to be 31.57%, 25.11% and 19.74% with 5%, 10% and 15% TWG respectively in simulated intestinal fluid after 5 hours. When the dissolution medium was replaced by simulated colonic fluid (pH 6.8) the drug release was found to be 85.46%, 72.82% and 57.24% after 8 hours for 5%, 10% and 15% TWG respectively.

3.4. In vitro drug release studies in simulated gastric fluid

In vitro drug release study of uncoated Tinidazole alginate microbeads and Eudragit coated microbeads was performed in pH progression medium at 37±0.5 ℃. The results showed 97.61±3.06 (FT1) of the drug release in the initial 4 to 5 hours in case of uncoated calcium alginate beads. This situation is not acceptable for drugs that are required to be released locally in the colon. Calcium alginate-pectinate beads were coated with Eudragit S100 polymer to retard the release of the drug.

The effect of concentration of sodium alginate, pectin, calcium chloride and cross-linking time on in vitro drug release was also studied. In vitro drug release after 5 hours was found to be 97.61±3.06% in the case of calcium alginate beads (FT1) while it was 78.89±2.39% for calcium alginate-pectinate beads (FT4) respectively. The total drug release in case of alginate pectinate beads was found to be lesser than that of the alginate beads which may result in incorporation of pectin within the alginate net in the microbeads resulting into constant release of the drug from the microbeads (FT4) for sufficient duration, which is required for successful delivery of drug to the colon.

The initial release of Eudragit S100 coated Tinidazole microbeads was found to be low. After 2 hours, small amount of Tinidazole could be estimated in pH 1.2 medium. Only 3.92 ±0.93% drug was released after 3 hours and 11.49±1.04% after 4 hours. After 8 hours, approximately 72.82±0.64% drug had been released in PBS (pH 6.8) (Figure 2). Eudragit S100 coating was done to protect the drug from acid and enzymes in gastric juice present in upper gastrointestinal tract to achieve successful colonic delivery.

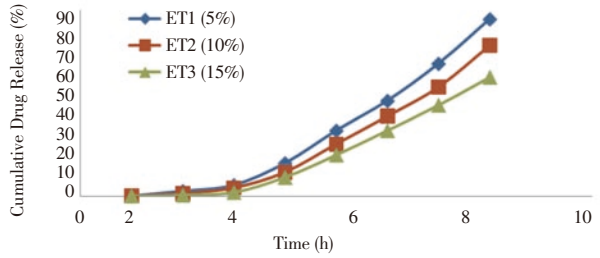


Fig. 2: In vitro dissolution profiles for Tinidazole from Eudragit S100 (5%, 10% and 15%) coated containing calcium alginate pectinate microbeads.

3.5. In vitro Drug Release Study in the Presence of Rat Caecal Contents

The in vitro drug release of Tinidazole from calcium alginate-pectinate beads and Eudragit S100 coated microbeads in the presence of 2% w/v rat caecal content in simulated colonic fluid showed faster drug release at different time periods compared with release study without rat caecal content. The cumulative percent drug release without rat caecal contents was found to be 56.91±2.53% and was higher in the presence of rat caecal content at 70.73±1.91% as shown in Figure 3.

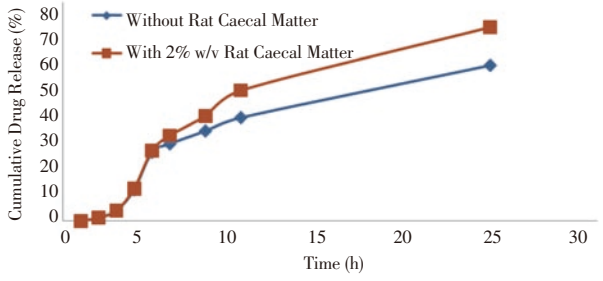


Fig. 3: Comparative percent drug release in Simulated Colonic Fluid (SCF pH 6.8) from Eudragit S100 coated microbeads of Tinidazole with and without rat caecal content

3.6. Mechanism of Drug Release

Data of in vitro release was fitted into various kinetic models to explain release kinetics of Tinidazole from microbeads. The kinetic models used were zero-order, Higuchi and Korsmeyer-Peppas model (Table 3). The interpretation of data was based on the value of the resulting regression coefficients.

Table 3

Release Kinetics of Optimized Formulations

Zero order		Higuchi Model		Korsmeyer Peppas Model	
R2	K	R2	K	R2	n
0.939	10.52	0.861	39.17	0.940	2.302

4. Discussions

The calcium alginate-pectinate microbeads (FT4) exhibited rough surface which may be due to presence of pectin which has low degree of esterification with sodium alginate. The carboxyl group of pectin and sodium alginate cross-linked with divalent calcium ion causes gelation to form microbeads. Moreover, the dispersion of Tinidazole as fine crystalline particles was also observed on the surface of microbeads. Formulation FT1 showed faster in vitro release as compared to FT4, due to the presence of large internal pores. Coated calcium alginate-pectinate microbeads displayed smoother surface.

The formulation with coating thickness of 10% TWG was found to be the most suitable due to its optimum drug release by virtue of ionization, disruption, and dissolution of the coating, compared to beads with 15% TWG coating which showed hindered drug release. Ideally the coating should be thick enough to resist the drug release for a sufficient period equivalent to transit through the upper GIT, yet not so thick so as to hinder drug release under colonic conditions. Coating thicknesses of 10% TWG appeared to fulfill these criteria.

The release kinetics of Tinidazole follows zero order as highest linearity was displayed for this curve. The Korsmeyer-Peppas release exponent n for the optimized formulation was 2.302 indicating release governed by Case-II transport or typical zero-order release.

The dosage form which enables site specific delivery of Tinidazole, with reduced side effects, holds much potential. The results of release studies indicate that Eudragit S100 coated microbeads deliver most of the drug in the colon and ensure its potential as an effective colon targeted delivery system.

Conflict of interest statement

We declare that we have no conflict of interest.

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Declaration of interest: The authors report no declarations of interest.

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