Research Article

Design and Evaluation of Colon Specific Drug Delivery System Containing Secnidazole Microsponges

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ABSTRACT

The aim of this study was to design colon targeted drug delivery system for secnidazole (SDZ) microsponges. The microsponges (MS) were formulated based on 22 full factorial design. The effect of independent variables i.e. inner phase solvent volume and polymer content on entrapment efficiency and in-vitro drug release was determined. The optimized microsponge formulation MS2 was developed into compression coated tablet by using xanthan gum and chitosan as a compression coating carrier. The compression coated formulations released less amount of SDZ in the physiological environment of the stomach and small intestine. When the dissolution study was carried out in presence of rat caecal content, it showed that SDZ compression coated tablet formulation CCT1 released almost 98.11% of drug at the end of 24 hr.

Keywords: Microsponge; Secnidazole; Colon; Amoebiasis.

INTRODUCTION

Amoebiasis is a disease caused by the parasite *Entamoeba histolytica.* It is more prevalent in individuals living in tropical regions with bad sanitary circumstances. Amoebiasis is a common infection of the human gastrointestinal tract. It is more closely related to poor sanitation and socioeconomic status.[1] Such disorders are treated with certain nitroimidazoles, such as secnidazole, tinidazole, ornidazole and metronidazole which are the drugs of choice. Secnidazole is the new version of nitroimidazole used in colonic infection management. Nonetheless, oral administration of secnidazole associated with certain side effects such as dizziness, headaches and digestive *disorders such* as vomiting, anorexia, glossitis and abdominal pain. These can be minimized by site-specific drug delivery with minimal systemic absorption. Due to the absorption/degradation of the active ingredient in the upper GIT, traditional oral dosage forms are unsuccessful in delivering drugs to the colon.[2]

The oral colon targeted drug delivery system must shield the drug from release into the stomach and small intestine, which enables a higher concentration of the drug to enter the colon with limited systemic absorption. [3]

Multiparticulate system can control the release of drugs in a variety of ways, such as rate control or site control. Microsponges are spherical, have good flow properties and better compression property provided by their microstructure. The reason for using MS as a colonic delivery system is that the drug carrier system has a size less than 200µm that can be effectively taken up by the macrophages present in the colonic tissue. Thus, MS exhibit effective localized drug action at the desired site.[4] In general, four primary approaches have been proposed for colon specific drug delivery, namely prodrugs, pH-dependent systems, time-dependent systems and microflora- activated systems. Every system has advantage as well as shortcoming.

The aim of this research was to design a new colon-specific drug delivery system containing SDZ microsponges. In order to provide colon-specific drug delivery, two different approaches were explored. In the first, MS core tablet was prepared and prepared core tablets were compression coated with a mixture of chitosan and xanthan gum that was found to be resistant to gastric and intestinal fluids but damaged by colonic microbial flora. As formulation with a single polymer in the coating layer was unsuitable for targeting drug release in the colon region, combination of chitosan with xanthan gum was used that exhibit control over SDZ release.

MATERIALS AND METHODS

Materials

Secnidazole was gifted by Aarati Drugs, Mumbai. Eudragit RS100 was procured from Evonic India pvt. ltd., Mumbai. Polyvinyl alcohol was procured from Loba Chemie pvt.ltd., Mumbai. Chitosan was gifted by CIFT Cochine. Xanthan gum was gifted by Arihant Innochem Pvt. Ltd. Mumbai. Sodium Starch Glycolate and Microcrystalline Cellulose was kindly gifted by Maple biotech pvt.ltd., Pune. Other chemicals and solvents were of analytical grade.

Fabrication of SDZ microsponges

The microsponges containing SDZ were fabricated based on 2^2 full factorial design (Table 1). Four experimental formulations (MS1-MS4) were prepared by varying volume of DCM and Eudragit RS100 at two levels and experimental trials were performed using Design Expert Software 8.0.7.1 (Stat-Ease, Inc., Minneapolis, USA). The dependent variables were entrapment efficiency and drug release at 8 hr. The SDZ microsponges were prepared by the quasi emulsion solvents diffusion method. Firstly, inner phase was prepared by dissolving Eudragit RS100 in

dichloromethane, then SDZ was added to it. Then the outer phase i.e. aqueous PVA solution was prepared separately. Inner phase solution was then poured into the PVA solution with stirring rate at 1000 rpm for 60 minutes. The blend was filtered to separate the MS and then placed in the desiccator.[5]

Table 1: Composition of SDZ microsponges

Constituents	Microsponge Formulation			
	MS1	MS2	MS3	MS4
Inner Phase				
Secnidazole (mg)	400	400	400	400
Eudragit RS100(mg)	400	400	800	800
Dichloromethane(ml)	5	7	5	7
Outer Phase				
Distilled water(ml)	200	200	200	200
PVA(mg)	50	50	50	50

Evaluation of SDZ microsponges

Particle size analysis

Microsponges were analyzed for particle size and size distribution using SAGLO Digital Microimaging Adapter, Saglo software version 1.0.

Drug content and encapsulation efficiency

In a 100 mL solution of pH 7.4 phosphate buffer saline (PBS), an accurately weighed quantity (100 mg) of MS formulation was distributed. The sample was ultrasonicated for 3 consecutive periods of 5 minutes, with a resting period of 5 minutes each. It was kept to equilibrate for 24 hrs at room temperature, then the suspension was centrifuged for 15 min at 3000 rpm. The supernatant was then diluted with 7.4 PBS and analyzed for concentration of secnidazole using a UV-visible spectrophotometer at 320 nm.[6]

In-Vitro drug release study

In-vitro release from SDZ microsponges was explored. Microsponges were precisely weighed and spread gently over the 900 mL surface of the dissolution medium (simulated gastric fluid, SGF). The contents were rotated with speed of 100 rpm at 37±0.5°C. Gastrointestinal transfer condition simulation was accomplished by changing the pH of the dissolution medium at separate periods of time. The pH of dissolution medium was preserved with 0.1N HCl at 1.2 pH for 2 hr. Then the dissolution medium was supplemented with KH_2PO_4 (1.7 g) and $Na_2HPO_4 \bullet 2H_2O$ (2.2 g) and the pH was adapted to 7.4 with 0.1N NaOH and kept up to 8 hr. Samples were withdrawn from the dissolution medium at multiple time intervals and evaluated using UV-vis spectrophotometer (JASCO V730) at 276 and 320.5 nm. During the study period, perfect sink circumstances were prevailed.[7]

Scanning Electron Microscopy

Microsponges surface morphology was investigated using Scanning Electron Microscopy (SEM). The sample was prepared for the SEM study by sprinkling the formulation lightly on a double adhesive cloth that was stuck to an Al stub. Using a gold sputter module in an elevated vacuum evaporator, the stubs were then covered with gold under an Argon atmosphere (VEGA3 TESCAN).[8]

Differential scanning calorimetry (DSC)

Thermal assessment of pure drug as well as optimized MS formulation was carried out using differential scanning calorimeter (Mettler Toledo DSC822e, USA). Sample (about 5 mg) was weighed precisely & then sealed into aluminum pans. All specimens were placed in the atmosphere of nitrogen at a heating speed of 10°C / min along a temperature at 25–430°C.[9]

Preparation of colon-specific tablet formulations:

The core tablet of optimized MS was prepared containing 100 mg SDZ. The composition of core tablet is given in Table 2. Sodium starch glycolate, microcrystalline cellulose and magnesium stearate were mixed properly and then mixture was directly compressed by applying force of 4000 Kg by the using round flat punches of 6 mm on Ten station rotary tablet punch machine.

Chitosan and xanthan gum were used as an outer shell for the compression coating of core tablet of SDZ microsponge (Table 3). Fifty percent of the coat weight was put in the die cavity; the core tablet was centered and the rest of the coat weight was added. At an applied pressure of 5000 kg, the coating material was compressed around the core tablet using round flat punches of 11 mm on the same tableting machine.[10]

Table 2: Composition of the core tablet of microsponge

Ingredients	Quantity (mg)
SDZ microsponges (eq.to 100mg of drug)	211
Sodium starch glycolate	20
Microcrystalline cellulose	24
Magnesium stearate	2.5
Talc	2.5
Total weight of tablet (mg)	260

Table 3: Composition of the compression coating mixture

	Formulation		
Ingredients (mg)	**CCT1**	**CCT2**	**CCT3**
Chitosan	202.50	67.50	135
Xanthan gum	67.50	202.50	135
Microcrystalline cellulose	25	25	25
Magnesium stearate	2	2	2
Talc	3	3	3
Total	300	300	300

In -vitro Evaluation

Preparation of rat caecal content medium

Wistar rats weighing 150-200g which were kept at normal pelleted diet, were used for the study. 1 mL of 2 % w/v chitosan aqueous dispersion was directly administered to rats for seven days daily for the purpose of inducing enzymes acting specifically on chitosan.

The rat caecal content medium was prepared as described previously by Rama Prasad et al., 1998. Thirty-minute before the drug release study, three rats were sacrificed by spinal traction. The contents from caecal bags were individually pooled, weighed, and then dispersed in the buffer to give 2 % w/v dilution. [11-12]

Drug release study was carried out to check the ability of chitosan and xanthan gum to release the drug in the presence of rat caecal content medium. The dissolution study of the compression coated tablet was performed in a USP basket type apparatus containing 900mL SGF, pH 1.2 maintained at 37±1°C for the initial 2hr, followed by SIF, pH 7.4 for 24hr. At specific time intervals sample was withdrawn and assayed spectrophotometrically at 276nm and 320.5nm in SGF & SIF respectively.

Stability Study

The stability of compression coated tablet (CCT1) was carried out as per ICH guidelines in accelerated conditions.The formulation was kept at 40℃ ± 2℃ and 75% ± 5% RH for three months. After 3 months tablets were analyzed for physical appearance and *in-vitro* drug release.[13]

RESULTS AND DISCUSSION

Characterization of the pure drug

The melting point of SDZ was found to be 75℃. Differential Scanning Colorimetry thermogram of SDZ has shown a sharp endothermic peak at 75°C corresponding to the melting point of drug, reflecting drug purity and crystalline form. (Figure not shown)

Evaluation of microsponges

Values of drug content, percentage yield, and encapsulation efficiency of different batches of drug loaded MS are shown in Table 4. The production yield was found between 63.01-67.91% for MS1-MS2 and 68.53-72.06% for MS3-MS4. The actual drug content of MS varies from 17.59 ± 0.20 to 33.07 ± 0.08. The particle size was influenced by varying polymer concentration. The mean particle size was found in the range of 137 ± 0.11 to 153 ± 0.39. It was observed that polymer concentration and volume of DCM have a significant effect on entrapment efficiency. The entrapment efficiency was found to be increased with an increase in the volume of DCM that may be observed due to the higher solubilization of drug in the organic solvent. Entrapment efficiency was also found to be increased with a decrease in particle size. The entrapment efficiency was found in range of 47.68-66.15% for MS1-MS4. The *in-vitro* release data revealed that 29.18 - 45.86% of drug was released after 8hrs. The drug release profile of microsponges MS1-MS4 batches was found to be significantly affected by the polymer concentration. Formulations MS1-MS2 showed higher drug release as compared to MS3-MS4 due to the lower concentration of polymer. It was also observed that the MS were capable of restricting drug release in GI fluids.

Table 4: Evaluation parameters of SDZ microsponges

Parameters	Formulations			
	MS1	**MS2**	**MS3**	**MS4**
Production yield (%)	67.91	63.01	72.06	68.53
Theoretcal drug content (%w/w)	50	50	33.33	33.33
Actual drug content (%w/w)	30.73±0.09	33.07±0.008	15.89±0.10	17.59±0.20
Partical size (µm)	144±0.08	137±0.11	153±0.39	150±0.27
Drug entrapment efficiency (%)	61.47±0.10	66.15±0.12	47.68±0.09	52.77±0.07
%CDR (after 8hr)	40.93±0.35	45.68±0.29	29.18±0.08	34.12±0.26

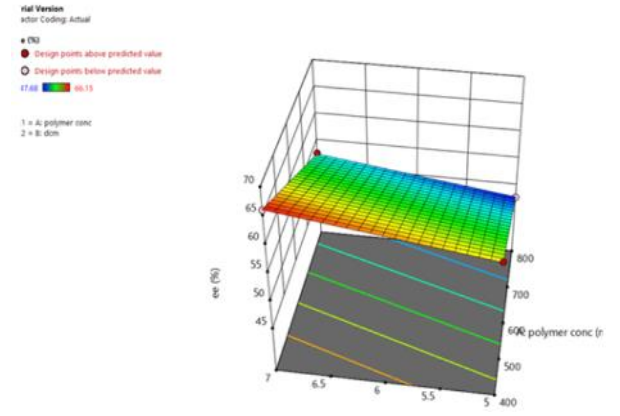
Statistical analysis was performed by the Design expert software trial version and polynomial equations were obtained. The equations transformed are:

% Entrapment efficiency (Y_1) = +57.02-6.79*X_1+2.44*X_2 ----(1)

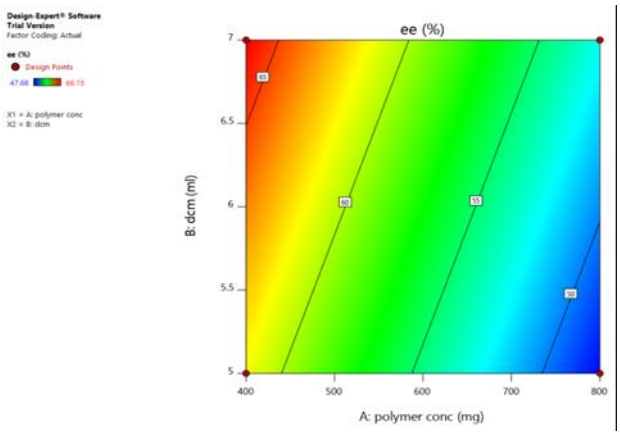
% Drug release (Y_2) = +37.48-5.83*X_1+2.42*X_2 ----(2)

Where X_1 and X_2 are independent variables. Figure 1 illustrates the impacts of independent variables i.e. polymer content (X_1) and volume of Dichloromethane (X_2) on dependent variables i.e. % entrapment efficiency and % drug release. Figure 1 (a), (b) depicts that polymer concentration has negative effect on entrapment efficiency. Moreover, as the amount of DCM increases, the entrapment efficiency also increases. Figure 1 (c), (d) depicts that as the concentration of polymer increases the drug release decreases while increase in DCM, increases the drug release. MS2 formulation was identified as an optimized formulation based on highest entrapment efficiency (66.15±0.12 %) and % cumulative drug release (45.68±0.29 %).

The microsponge formulations were visualized by scanning electron microscope (SEM) to assess the morphology of MS and surface. The representative SEM photographs of MS are shown in Figure 2. It was observed by SEM analysis that MS were finely spherical and uniform with a highly porous framework (Figure 2a, 2b). The microsponges are sufficiently resilient to resist the pressure applied during tablet compression. Even after compression as a tablet, it did not demonstrate any sign of disruption (Figure 2d). This has been confirmed by micro photographs of broken tablet. The thermogram of MS formulation showed suppressed melting endotherm because of partial protection of SDZ due to MS encapsulation as well as altered crystallinity of SDZ in MS formulation confirming its dispersion in system (Figure Not shown).



(a)



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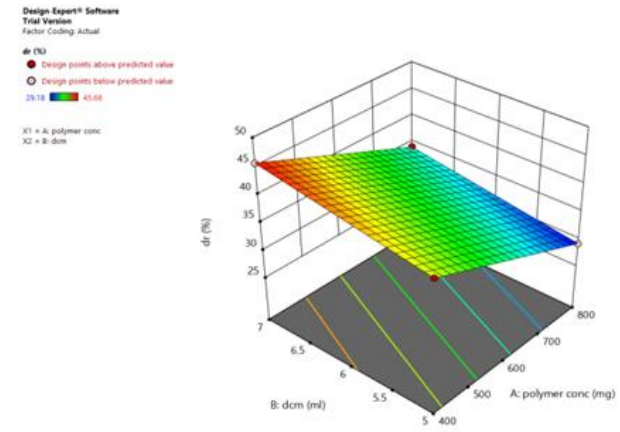
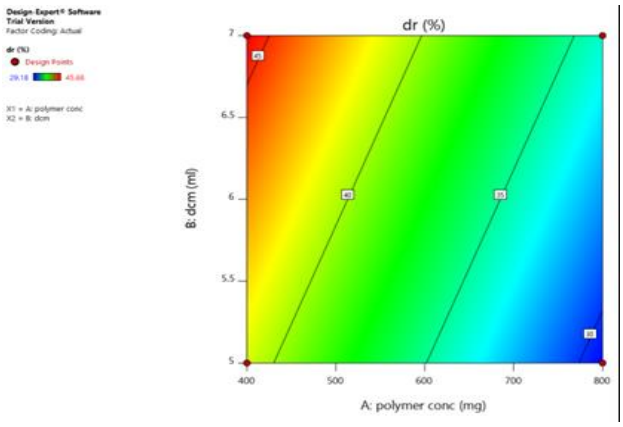
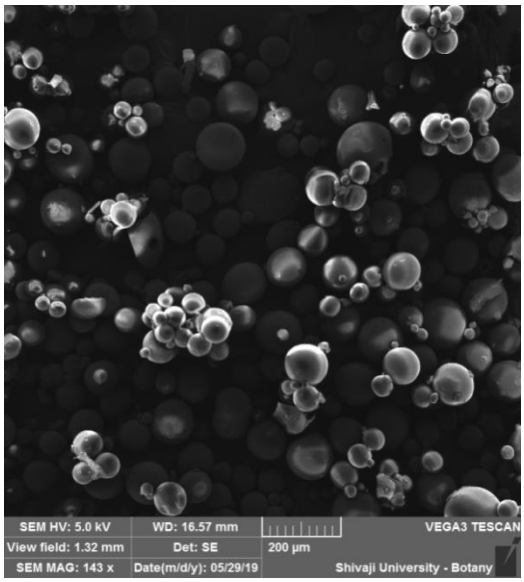


(c)

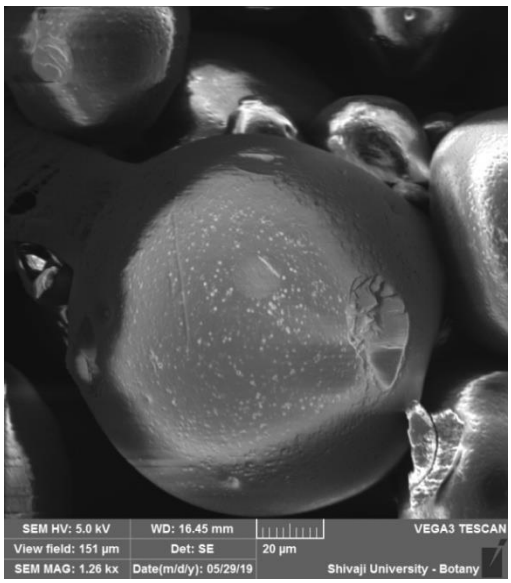


(d)

Figure 1: Response Surface Plot for the effect of independent variables on a) % entrapment efficiency c) % drug release; Contour plot showing the effect of independent variables on b) % entrapment efficiency (d) % drug release.



(a)



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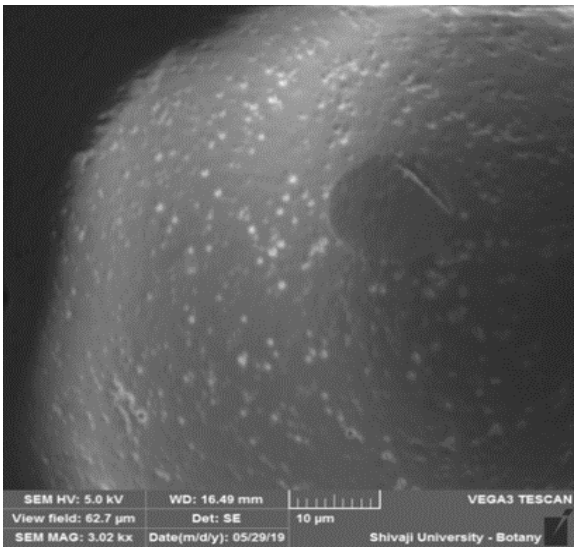

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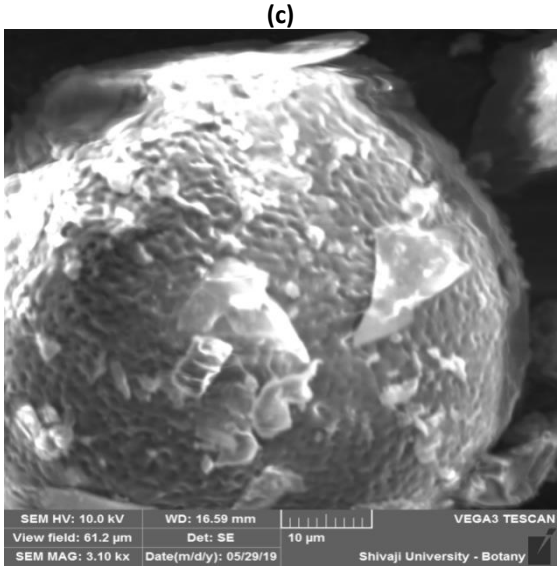

(d)

Figure 2: Scanning Electron Microphotographs of optimized MS formulation. (a) and (b) represents the whole image of MS, (c) represents surface photographs shows a porous structure, (d) represents the structure of MS after tablet compression

Evaluation of core and compression coated tablet

The microsponge formulations showed highly porous spherical structure that produces a mechanically strong tablet. For the preparation of compression coated tablet core tablet was prepared. The results of thickness, hardness, friability, weight variation and drug content of the core tablet and the compression coated preparation are given in Table 5. The friability of all formulations indicated that the tablets could resist the usual mechanical stress during handling.

The time required for disintegration of core tablet was evaluated and disintegration was found within 2 minutes. As the core tablet is anticipated to release the drug entirely in the colon region, the quicker disintegration was required. The quicker tablet disintegration could be explained for two reasons: first, it was due to the incorporation of sodium starch glycolate as a super disintegrant in the formulation. Sodium starch glycolate quickly absorbs dissolution fluid followed by fast and enormous swelling causing quicker tablet disintegration.[14-15] Second, tablet hardness is low indicating loosely compressed core tablet. The results of the weight variation test depicted that the percentage deviation of tablet weights was within the acceptable limits as per Indian Pharmacopoeia.

Hardness of the prepared formulations was found in the range of 6.71±0.042 to 6.83±0.026 kg/cm^2 indicating that tablets with sufficient hardness could be prepared using the selected polymers. Marketed tablet formulation showed 50. 14% of drug release in 6 hr. The colon-oriented drug delivery systems should not only safeguard the drug from release in the physiological environment of the stomach & small intestine but should also release the maximum amount of drug in the colon. Therefore, *in vitro* drug release comprising 2 % w/v of rat caecal content was performed in SIF, which was added to SIF after 6 hours of dissolution study. Three compression coated formulations were compared with each other, with and without rat caecal content. In SIF after 24 hrs, it gives 88.1±0.45%, 75.32±0.55% and 79.81±0.61% of drug release respectively in the absence of caecal content media (Data not shown). In the presence of caecal content, drug release from formulations was found to be enhanced. At the end of the dissolution study, the SDZ compression-coated formulation CCT1 released nearly 98.11 % of its drug in the physiological environment of colon (Figure 3).

Table 5: Evaluation study of core and compression coated tablet

Formulation	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)	Weight variation (mg)	% Drug content (%w/w)
Core tablet	3.51±0.063	2.71±0.045	0.73±0.038	261.5±0.12	96.58±0.16
CCT1	4.14±0.030	6.71±0.042	0.45±0.025	562.5±0.47	97.53±0.68
CCT2	4.52±0.383	6.74±0.095	0.35±0.060	564.5±0.35	96.52±0.43
CCT3	4.30±0.09	6.83±0.026	0.44±0.048	563.2±0.53	99.63±0.59

All values are expressed as mean ±SD, n=3

Stability Studies

The CCT1 formulation was subjected to 3-month stability study at accelerated condition and was analyzed for physical appearance and *in vitro* drug release. After 3 months the formulation was found to show no change in physical appearance and *in vitro* drug release. Thus, all these parameters suggested that the formulation CCT1 may have good shelf life.

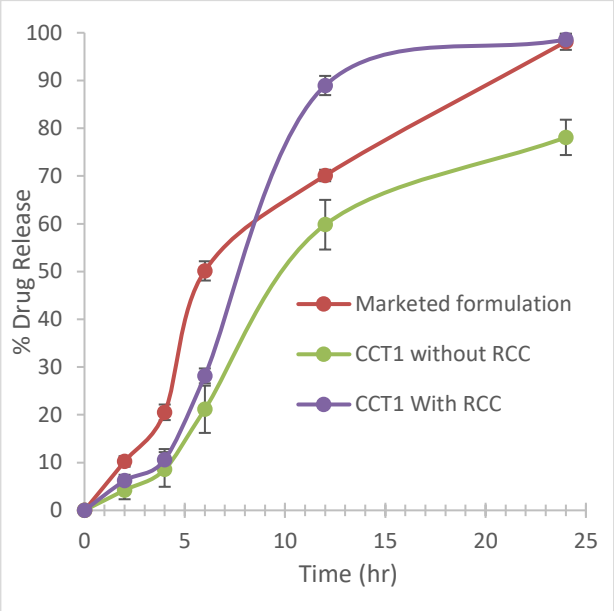

Figure 3: *In-vitro* drug release profile of marketed formulation and compression coated tablets CCT1 with and without rat caecal content.

CONCLUSION

This study presents is a new approach for modification of MS with great potential for colonic drug delivery. Microsponges showed a unique compressibility character offering a new way of producing mechanically strong tablet owing to plastic deformation of the sponge-like structure. The colon targeted tablet formulation including MS was prepared based on approach of triggering mechanism of microflora activation, which gives delivery of the drug to the proximal colon with the minimum release in the stomach and small intestine. The microsponges i.e particulate form has been used to provide a more uniform distribution of the drug in the colon and provide efficient local action. The obtained MS showed a spherical shape with a highly porous nature. The drug release from MS and core tablet was found to be rapid as compared to the compression coated tablet. The compression coated tablet prepared using natural polysaccharide chitosan and xanthan gum at the different ratio that protects the drug from being released in the stomach and small intestine. Thus, it was concluded that MS based colon targeted tablet approach was the potential system for colonic delivery of secnidazole.

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