Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue S- 3 Year 2024 Page 195-202

Development And Characterization Of Colon Targeted Microspheres For Intestinal Infection

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Abstract

Various colon-targeted oral delivery systems have been explored so far to treat colorectal diseases, including timed-release systems, prodrugs, pH-based polymer coatings, and microflora-triggered systems. The polysaccharide-based colon-targeted delivery system has been found to be quite promising as polysaccharides remain unaffected by gastric as well as upper intestine milieu and are only digested by colonic bacteria upon reaching the colon. The metronidazole containing microsphere has other characterization is in the term of percentage yield, entrapment efficiency, FT-IR and the in-vitro drug release. The In-vitro release pattern of metronidazole studied in colonic environment as 900ml media using USP dissolution apparatus II. The cumulative release rate of prepared microspheres was followed by a sustained release and fitted for classic Higuchi kinetic model. The results showed that chitosan microspheres are thought to have the potential to maintain drug concentration within target ranges for a long time, decreasing side effects caused by concentration fluctuation, ensuring the efficiency of treatment and improving patient compliance by reducing dosing frequency.

Keywords: Metronidazole, mucoadhesive microspheres, polysaccharide polymers, colon targeted drug delivery system

Introduction:

The drug-delivery system should deliver drug at a rate dictated by the needs of the body over a specified period of time. The goal of any drug delivery system is to provide a therapeutic amount of drug to a proper site in the body, so that the desired drug concentration can be achieved promptly and then maintained. The idealized objective points to the two aspects most important to drug delivery, namely, spatial placement and temporal delivery [1-2]. A dosage form that allows at least a twofold reduction in dosage frequency as compared to that drug presented as an immediate-release (conventional) dosage form. Examples of extended-release dosage forms include controlled-release, sustained-release, and long-acting drug products [3]**.** Most of the drugs introduced to clinical medicine exert their effects by interactive interference with cell and cell membrane related structure and functions through concentration dependent reversible interactions at specific receptor site.

Obviously, to obtain a desirable therapeutic response, the correct amount of drug should be transported and delivered to the site of action with subsequent control of drug input rate. The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Ehrlisch, who proposed drug delivery to be as a "Magic Bullet". Targeted-release drug products are a type of dosage form that releases drug at or near the intended physiologic site of action. Targeted-release dosage forms may have either immediate- or extended-release characteristics [4]**.** The controlled-release formulations for oral drug delivery are diffusion-controlled systems; solvent activated systems, and chemically controlled systems. Diffusion-controlled systems include monolithic and reservoir devices in which diffusion of the drug is the rate-limiting step, respectively, through a polymer matrix or a polymeric membrane. Chemically controlled systems release drugs via polymeric degradation (surface or bulk matrix erosion) or cleavage of drug from a polymer chain [5]**.** Colon specific drug delivery systems are designed to obtain targeted drug delivery to the large intestine (colon). They provide local delivery for the treatment of colonic diseases like inflammatory bowel disease (ulcerative colitis and crohn's disease) and colon cancer, where it is necessary to attain high concentration of the drug. These systems are also useful for delivery of therapeutic peptides and proteins, which are other wise degraded and / or poorly, absorbed in the stomach and small intestine but may be better absorbed from the colon. There are mainly four strategies are currently being persued to achieve drug release specifically in the colon. pH controlled approaches is the fact that the luminal pH of the healthy distal colon is slightly higher than that of the proximal small intestine has led to the development of oral dosage forms that are intended to release the drug at the colonic pH. Several commercial drug formulations designed for colon-specific drug delivery rely on the physiological difference between the luminal pH of the acidic stomach and that of the distal small intestine [6]. In the stomach pH ranges between 1 and 2 during fasting but increases after eating. The pH is about 6.5 in the proximal small intestine and about 7.5 in the distal small intestine. From the ileum to the colon pH declines significantly. It is about 6.4 in the ceacum. However, pH values as low as 5.7 have been measured in the ascending colon in healthy volunteers. The pH in the transverse colon is 6.6, in the descending colon 7.0 pH. There are pH controlled drug delivery systems is formulated by two method [7]. Formulation coated with enteric polymers releases drug when pH moves towards alkaline range. Degradation of pH-sensitive polymer in the gastrointestinal tract releases the embedded drug [8]. Enzyme controlled drug release relies on the existence of enzyme-producing microorganisms in the colon. The colonic microflora produces a variety of enzymes, including azoreductase, various glycosidases and, at a lower concentration, esterases and amidases, that can be exploited for colon-specific drug delivery [9]. Sustained release of drugs at colonic site can be useful in the treatment of certain diseases. Colon was found to be a promising site for systemic absorption of peptides and proteins, because the less hydrolytic hostile environment is present in comparison with stomach and small intestine as well as the existence of specific transporters. The colon is a highly responsive site for the absorption of poorly absorbable drugs. The treatment of colon diseases such as ulcerative colitis, colorectal cancer and Crohn's disease is more effective with direct delivery of drugs to the affected area. Drug action can be improved by developing new drug delivery system, such as the mucoadhesive microsphere drug delivery system. These systems remain in close contact with the absorption tissue, the mucous membrane, releasing the drug at the action site leading to a bioavailability increase and both local and systemic effects. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity. Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 μm range in diameter having a core of drug and entirely outer layers of polymer as coating material. This can be achieved by coupling bioadhesion characteristics to microspheres and developing "mucoadhesive microspheres". Mucoadhesive microspheres have advantages like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer and specific targeting of drugs to the absorption site [10]. Mucoadhesion is defined as the interaction between a mucin surface and a synthetic or natural polymer. Mucoadhesion has been widely promoted as a way of achieving site-specific drug delivery through the incorporation of mucoadhesive hydrophilic polymers within pharmaceutical formulations such as "microspheres" along with the active pharmaceutical ingredient (API). Microspheres are defined as spherical particles having size less than 200μm and made up of polymer matrix in which therapeutic substance is dispersed throughout the matrix at the molecular or macroscopic level. The rationale of developing mucoadhesive microsphere drug delivery system lies behind the fact that the formulation will be 'held' on a biological surface for localized drug delivery. The API will be released close to the site of action with a consequent enhancement of bioavailability [11-12]. Metronidazole is a prodrug; it requires reductive activation of the nitro group by susceptible organisms. Its selective toxicity toward anaerobic and microaerophilic pathogens such as the amitochondriate protozoa *T. vaginalis*, *E. histolytica*, and *G. lamblia* and various anaerobic bacteria derives from their energy metabolism, which differs from that of aerobic cells. These

organisms, unlike their aerobic counterparts, contain electron transport components such as ferredoxins, small Fe-S proteins that have a sufficiently negative redox potential to donate electrons to metronidazole. The single electron transfer forms a highly reactive nitro radical anion that kills susceptible organisms by radical-mediated mechanisms that target DNA and possibly other vital biomolecules. Metronidazole is catalytically recycled; loss of the active metabolite's electron regenerates the parent compound. Increasing levels of O_2 inhibit metronidazole-induced cytotoxicity because O_2 competes with metronidazole for electrons generated by energy metabolism. Thus, O_2 can both decrease reductive activation of metronidazole and increase recycling of the activated drug. Anaerobic or microaerophilic organisms susceptible to metronidazole derive energy from the oxidative fermentation of ketoacids such as pyruvate. Pyruvate decarboxylation, catalyzed by pyruvate: ferredoxin oxidoreductase (PFOR), produces electrons that reduce ferredoxin, which, in turn, catalytically donates its electrons to biological electron acceptors or to metronidazole.

Material and Methods

Preparation of mucoadhesive microspheres: The microspheres of metronidazole drug were prepared by modified emulsion cross-linking method. The microspheres were prepared by using two different phases. One of the aqueous phase prepared by dissolving acrycoat S 100 as polymer in distilled water at 50°C. The drug was subsequently added to above prepared solution upto completely dissolving. Other was organic phase in the ratio of 50:50 w/w of petroleum ether and light liquid paraffin with required quantity of emulsifier span 80 / tween 80 as surfactant. Now, the prepared aqueous phase was again added to an organic phase, with constant stirring using a mechanical stirrer to form w/o type of emulsion. The cross-linking agent gluteraldehyde (1 ml) was added as to this solution after 10 min, at 40°C, with required stirring speed 500 rpm upto 3 h (Table 1) for individual formulation. The resulting microspheres were washed and filtered with n-hexane, dried under vacuum at 40°C for 1 h and stored in air tight container.

Polymeric coating of uncoated microspheres: The formulations containing chitosan / acrycoat S100 microspheres were coated by using pan coating technique with three successive layers. First layer over the core tablet was Eudragit E-100 polymer, second layer was HPMC coat as a barrier layer, and third layer or upper was Eudragit L-100 as enteric coated layer. The process parameter for coating was optimized and coating of different layer was done. The coating solution for different coating layer was prepared according to the formulae. Firstly the different process parameter for coating process was optimized and coating was done to develop colon specific drug delivery system **(Table 2-3)**.

Characterization of microspheres: The prepared chitosan / acrycoat S 100 mucoadhesive microspheres evaluated by such parameters i.e. Particle size analysis, Flow properties, Shape and Surface Characterization of Microspheres by Scanning Electron Microscopy, Percentage Yield, Drug Entrapment, In vitro swelling, In-Vitro drug release studies.

Particle size analysis: Particle size analysis plays an important role in determining the release characteristics of drug. The sizes of microspheres were measured by using an optical microscope, and the mean particle size was calculated by measuring nearly 100 particles with the help of a calculated ocular micrometer.

Flow properties: The flow properties of prepared microspheres were characterized for identification of flow character of powder in terms of carr's index, hausner's ratio and angle of repose. The Carr's index ((IC)) and Hausner's ratio (HR) of drug powders were calculating according to following equation:

Carr's Index (IC) = ρTapped - ρBulk / ρTapped
Hausner's ratio (HR) = ρTapped / ρBulk

The angle of repose (θ) was measured by fixed height method. This was calculated by following equation:

Angle of repose (θ) = $\tan^{-1}$ 2 H / D

Where H is the surface area of the free standing height of the powder pile and D is diameter of pile that formed after powder flow from the glass funnel

Scanning Electron Microscopy analysis for Shape and Surface Characterization: The shape and surface characteristics of the microspheres were observed by scanning electron microscopy. The freeze-dried

microspheres were coated with gold using a sputter coater (Agar sputter coater, Agar Scientific, Stansted, UK) under high vacuum microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology.

Percentage Yield: The prepared microspheres were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

$$\text{Percent Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} * 100$$

Drug Entrapment: The various formulations of the chitosan microspheres were subjected for drug content. 100 mg of microspheres from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved with 10ml ethanol in 100ml volumetric flask and makeup the volume with phosphate buffer pH 7.4 buffer. This resulting solution is than filtered through whatmann filter paper No. 44. After filtration, from this solution 10 ml was taken out and diluted up to 100 ml with phosphate buffer pH 7.4 buffer. Again from this solution 1 ml was taken out and diluted up to 10 m1 with phosphate buffer pH 7.4 buffer and the absorbance was measured at 320 nm against phosphate buffer pH 7.4 buffer as a blank. The percentage drug entrapment was calculated as follows.

$$\text{Percent Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} * 100$$

Swelling Index (%): The Swelling index of prepared chitosan drug loaded coated microspheres was determined by placing 100 mg of microspheres and in a cellophane membrane dialysis bag containing phosphate buffer (pH 7.4) dissolution medium. Then microspheres were allowed to swell for a period upto 12 h. The changes in weight were measured by removal of the samples and blotted with a filter paper for 10 sec to absorb excess solvent on surface. The degree of swelling was determined using the following equation:

$$Si = Wt – W0 / W0$$

where Si represents the degree of swelling, Wt and W0 represent weights of the sample at equilibrium swelling and the original dry weight, respectively

In-Vitro drug release studies: The dissolution study of prepared coated microspheres was kept in a USP paddle apparatus in different pH condition according to optimization of various coating layer at 50 rpm at 37 ± 0.5 °C. The polymeric matrix system was tested using 0.1N HCL pH 1.2 for 2 h. The dissolution of optimized formulation was carried out at pH 1.2 for 2 h followed by phosphate buffer pH 6.8 for 4 h and further continued in phosphate buffer pH 7.4 for 2 h. The samples were withdrawn at various time intervals and replaced with an equivalent amount of fresh dissolution medium. Dissolution samples were filtered through a whatmann filter paper and analyzed using a validated UV spectroscopy method. The absorbance of all samples were measured at 272 nm, 320 nm and 319 nm for 0.1 N HCl solution, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 respectively for drug metronidazole.

In vivo mucoadhesive Study: In vivo mucoadhesion behavior of the prepared colon targeted microspheres was observed through X-ray radoioscientigraphy images. The proposed colon targeted microspheres containing barium sulphate as placebo material other tan API was used as a diagnostic agent with polymeric composition of MCTM14 and MCTM16 for the justification of mucoadhesion behavior. The microspheres was prepared placebo without addition of drug with barium sulphate was filled in empty gelatine capsular shell. The colon targeted prepared microspheres in gelatine capsules were administered with 10 ml of water to a beagle dog after a light meal. The source of the X-ray machine and the animal were kept uniform throughout the procedure, and finally, images of the gastric region were captured at 0, 1, 3, 6 and 12 h to observe the mucoadhesion of microspheres.

Table 1: Formulation of drug loaded microspheres

F. Code	Drug (mg)	Acrycoat S 100	Emulsifier %		Stirring Rate (rpm)	Acid soluble coating (Inner layer)	Barrier layer coating (Middle layer)	Enteric coating (Outer layer)
			(Span 80)	(Tween 80)				
MCM1	100	500	5	0	500	5%	5%	10%
MCM2	100	1000	5	0	500	5%	5%	10%
MCM3	100	500	10	0	500	5%	5%	10%
MCM4	100	1000	10	0	500	5%	5%	10%
MCM5	100	500	0	5	500	5%	5%	10%
MCM6	100	1000	0	5	500	5%	5%	10%
MCM7	100	500	0	10	500	5%	5%	10%
MCM8	100	1000	0	10	500	5%	5%	10%

Table 2: Composition of coating solutions for coating on uncoated microspheres

S. No.	Ingredients	Quantity
Acid soluble coating (Inner layer)		
1	Eudragit E-100	5 g
2	Methanol	100 ml
Barrier layer coating (Middle layer)		
1	HPMC	5 g
2	Methanol	70 ml
3	Methylene chloride	30 ml
Enteric coating (Outer layer)		
1	Eudragit L-100	10 g
2	Methanol	90 ml
3	Glycerol (plasticizer)	10 ml

Table 3: The coating process parameters for the coating on uncoated microspheres

S. No.	Process Parameter	Parameter Used
1	Coating nozzle diameter (mm)	1
2	Spraying rate	4 ml/min
3	Pan Speed (RPM)	15-20
4	Inlet air temperature (°C)	60-70
5	Air Pressure (Kg/cm2)	2-2.5

Results and Discussion:

The particle size was calculated by measuring nearly 200 particles with the help of a calculated ocular micrometer. The result was varied mean particle size 138.07 – 261.18 µm. The shape and surface characteristics of the microspheres were observed by scanning electron microscopy. Microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology. The microspheres were shown rough surface structure and observe balloon like structure. The flow properties of prepared microspheres were characterized for identification carr's index, hausner's ratio and angle of repose. Flow properties of different batches of microsphere were have good to fair flow in characterstics because of rough surface structure of prepared microspheres. Percentage yield of different batches of microsphere have varied from 77.1 % - 97.7 %. The result was optimized that the coated microspheres have more percentage yield properties than the other ones. The various formulations of the acrycoat S 100 colon targeted coated microspheres were subjected for drug content. The percentage drug entrapment was calculated of different batches of microsphere were showed more drug loading capacity for coated microspheres varied from 78.38 % - 93.73 %. The swelling index was determined by allowing swelling for a period of 12 h. The changes in weight were measured and degree of swelling of different batches of microsphere was depending on the higher percentage of acrycoat S 100 polymer during the formulation and was varied from 11.11 % - 37.21 %.. Colon targeted metronidazole microspheres with acrycoat S 100 (MCTM8) with tween 80 as surfactant with 500 rpm

with 5 % eudragit E 100 as inner layer, 5% HPMC middle layer and eudragit L 100 coating showed simple release profile. The formulation release more than 99 % of the drug in gastric environment in controlled and sustained manner upto 12 h. Regression analysis was performed and the r^2 values suggested that the curves were fairly linear and slope values were computed from the graph. The release exponent "n" values were in the range of 1.1212 to 1.3219 for MCTM1 to MCTM8. For all of the batches the value of release exponent "n" was > 0.89 indicating Super-case II transport mechanism. The in-vitro drug release studies of uncoated and coated microspheres were conducted in a USP paddle apparatus in different pH condition for metronidazole. The in-vitro Release profile of microspheres was characterized for release percentage and release rate **k**. Release data within the linear range were selected and fitted to various mathematical model: The linear equation is based on regression of at least three release data, and only correlation coefficient of over 0.99 is acceptable.

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Table 1: The various physical parameters of different batches of colon targeted microspheres

F. Code	Mean particle size[b] (µm)	Bulk density[a] (g/cm^3)	Tapped density[a] (g/cm^3)	Carr's index[a] (%)	Angle of repose[a] (Θ^o)	Percentage yield	Percent entrapment	Degree of swelling
MCTM1	237.13 ±3.65	0.36± 0.028	0.42± 0.007	14.28 ± 0.25	25.22± 1.16°	87.56± 1.41	83.45± 1.89	1.32 ±0.25
MCTM2	241.16 ±2.16	0.35± 0.011	0.42± 0.011	12.16 ±0.01	31.02± 2.18°	92.85± 2.58	87.17± 1.05	1.73 ±0.54
MCTM3	248.93 ±3.92	0.40± 0.017	0.45± 0.012	11.11 ±0.97	24.91± 1.20°	87.95± 2.48	84.7±2. 13	1.34 ±0.64
MCTM4	246.28 ±4.38	0.32± 0.018	0.38± 0.017	11.14 ± 0.15	28.45± 1.56°	92.87± 2.54	88.7±1. 29	1.76 ±0.62

MC TM5	252.12 ±3.81	0.43± 0.011	0.49± 0.021	12.24 ± 0.18	23.25± 1.02°	88.87± 2.68	85.27± 1.41	8.76 ±1.22
MC TM6	251.15 ±3.87	0.49± 0.011	0.51± 0.011	12.00 ±0.38	27.54± 1.59°	95.2±2 .69	90.14± 1.14	1.79 ±0.55
MC TM7	258.13 ±3.51	0.42± 0.021	0.48± 0.024	12.51 ±0.27	23.01± 1.11°	80.1±1 .03	81.85± 2.06	3.11± 1.01
MC TM8	138.07 ±4.05	0.42± 0.028	0.55± 0.019	13.10 ±0.12	27.08± 1.63°	78.5±1 .01	86.18±. 74	1.93 ±0.94

Table 2: in-vitro Dissolution data of colon targeted microsphere of metronidazole (MCTM1 – MCTM8)

Time (h)	**MCTM 1**	**MCTM 2**	**MCTM 3**	**MCTM 4**	**MCTM 5**	**MCTM 6**	**MCTM 7**	**MCTM 8**
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	5.02	1.78	4.68	1.6	3.23	1.56	3.01	1.54
5	21.14	9.69	18.34	8.89	15.67	11.98	10.34	9.23
6	39.01	17.11	38.67	15.34	28.45	26.21	25.46	31.43
7	45.21	35.02	44.23	32.12	39.72	48.32	41.34	49.31
8	61.02	49.05	59.67	47.23	53.68	62.34	58.34	65.31
9	77.61	71.23	75.6	68.23	72.34	79.21	65.78	77.21
10	89.02	88.01	88	84.21	87.46	89.32	81.25	90.12
11	95.01	99.08	94.6	98.98	93.56	95.34	94.3	96.01
12	99.99	99.89	99.98	99.89	99.78	98.13	99.24	98.13

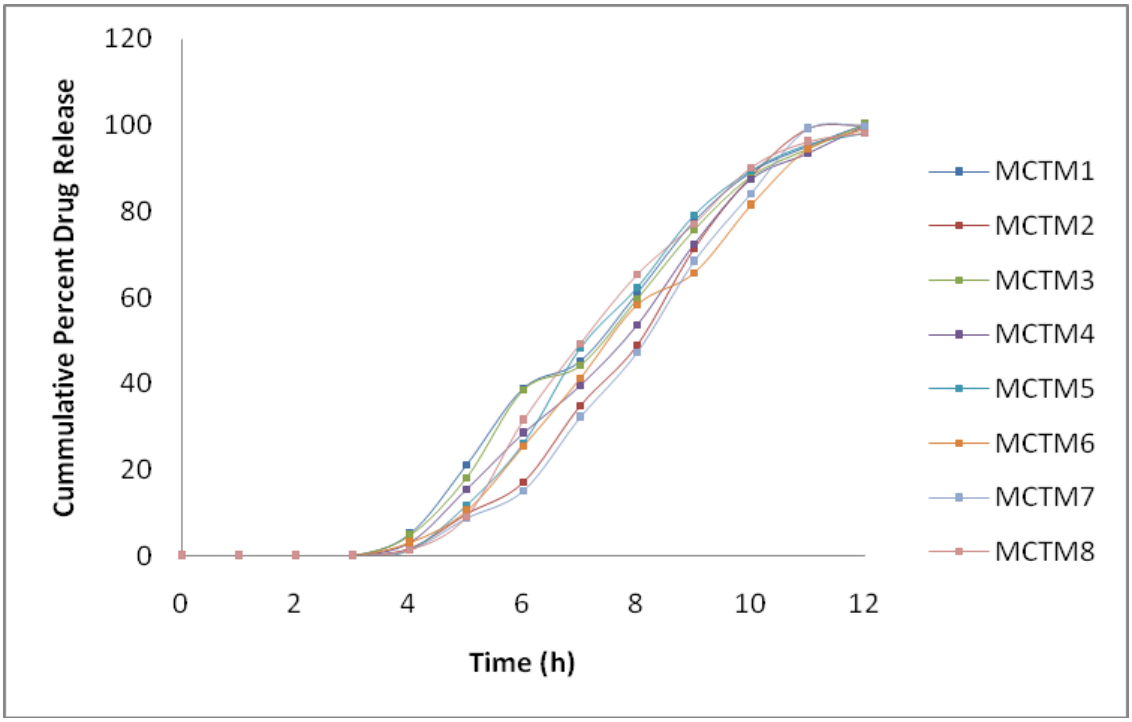

Figure 1: Zero-order plots of colon targeted microsphere of metronidazole (MCTM1 – MCTM8)

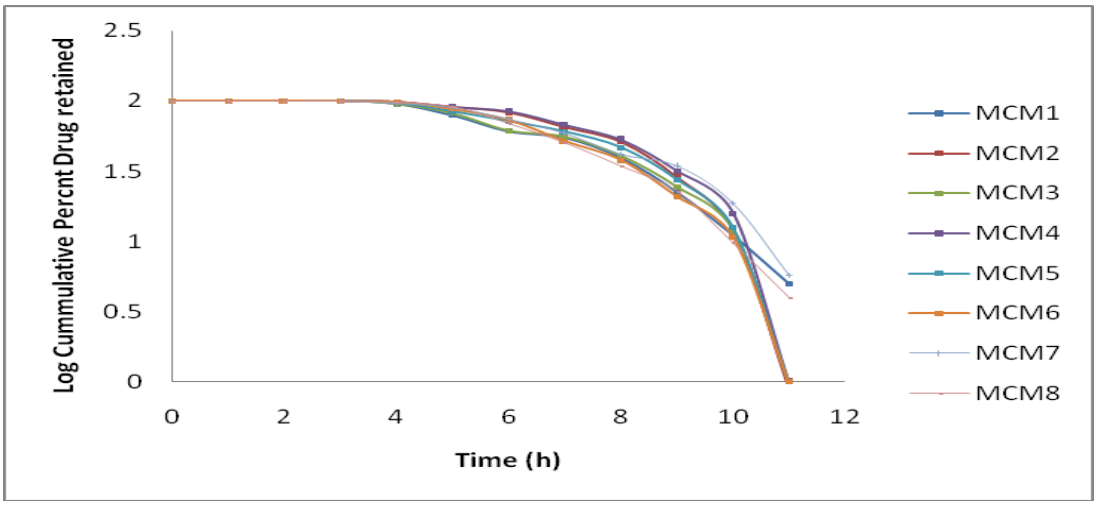

Figure 2: First-order plots of of colon targeted microsphere of metronidazole (MCTM1 – MCTM8)

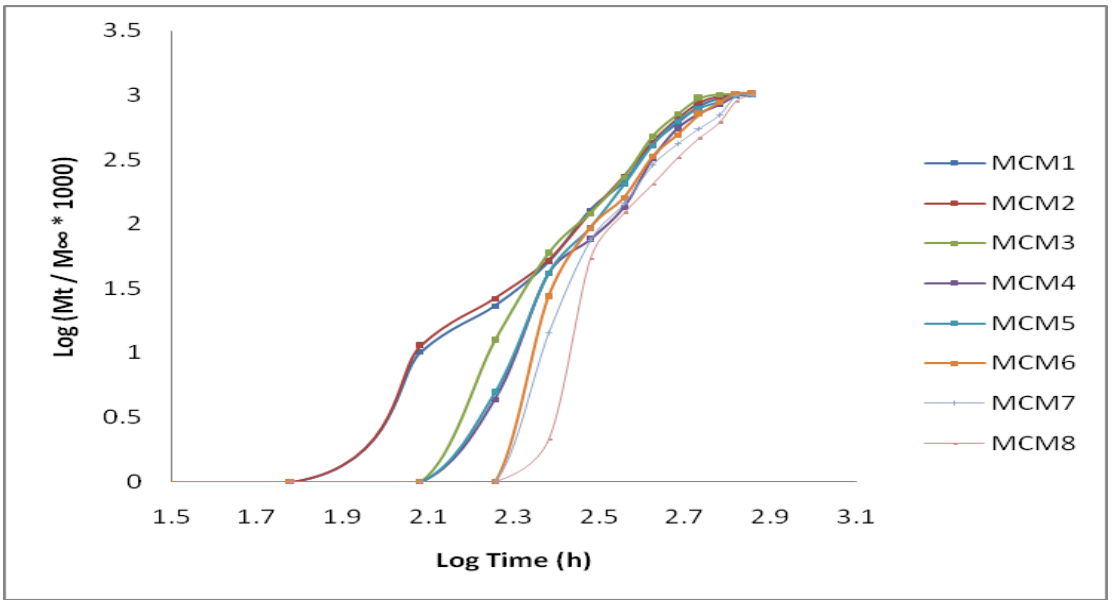

Figure 7.13: Korsmeyeyr's Peppas plots of colon targeted microsphere of metronidazole (MCTM1 – MCTM8)

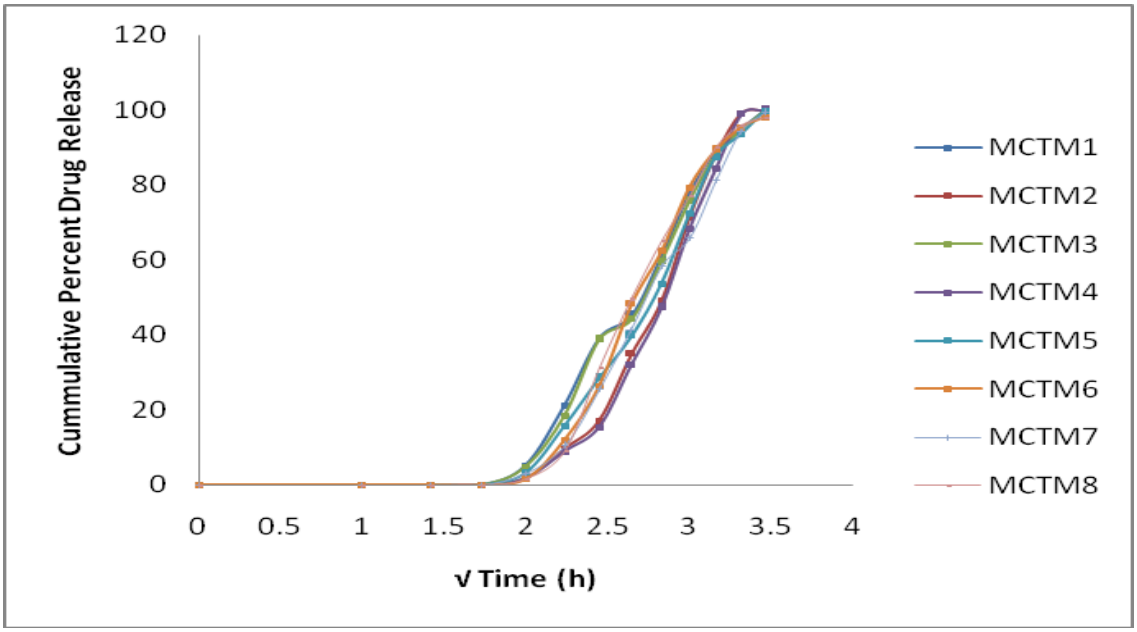

Figure 7.14: Higuchi plots of colon targeted microsphere of metronidazole (MCTM1 – MCTM8)