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Research Article

Formulation optimization and release kinetics of Metronidazole matrix, compression and spray coated tablets: Effect of organic acid on colon targeted drug delivery system

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ABSTRACT

Amoebiasis is an infection of the large intestine caused by *Entamoeba histolytica*, and it is mainly present in the intra-intestinal lumen. The efficient treatment of amoebiasis and other colonic infections could be achieved by targeting the drug to the colon. Metronidazole is the drug of choice for intestinal amoebiasis and other colon infections and the best approach for this drug is to target the drug delivery to colon which would make the drug effective with low dose and prevent the potential hazards observed in conventional dose. Moreover, addition of suitable organic acid in the formulation could enhance the drug solubility where less free aqueous is present than intestine. The aim of the present investigation was to formulate matrix formulations using different concentrations of guar gum and pectin with suitable organic acid and to prevent the premature drug release in the GI tract, the matrix formulations further taken for compression and spray coating to test the suitability for targeted drug delivery to the colon. The release kinetics of the formulations was performed using PCP-Disso Excel software. All the Matrix, Compression coated and Spray coated formulations were showed the desired physicochemical properties as per the official limits. Based on the drug release study in pH 1.2 (0.1N HCl), Phosphate buffer pH 7.4 and 6.8, the three MGT F8, MGTE F20, and MGTES F32 were found to be best and they were taken for further release study in phosphate buffer pH 6.8 with rat cecal content (4% w/v). The formulations MGTE F20 (r^2 - 0.9986, n - 1.0601) and MGTES F32 (r^2 -0.9974, n - 1.1501) were shown zero order release in terms of its kinetic release. Guar gum (40%) and Tartaric acid (80mg) was found to be suitable for this formulation and further gastrointestinal resistant coating is necessary for targeting the drug release. Hence, this special drug delivery system is needed to minimize the hazardous effects and to increase the effectiveness of the drug.

Keywords: Amoebiasis; Metronidazole; Organic acids; coated matrix tablets; release kinetics.

INTRODUCTION

Amoebiasis is an infection of the large intestine caused by *Entamoeba histolytica*, celled protozoan parasite. The current estimate is that *E*. *histolytica* causes between 34-50 million symptomatic infections each year and leading to the death of 40–100 thousands of people, which makes amoebiasis second to malaria as a cause of death resulting from protozoan parasite. The trophozoites of *E*. *histolytica* can invade the colonic epithelium, causing amoebic colitis (Haque R, et al. 2006; WHO, 1969; WHO, 1997; Mondal D, et al. 2006; Krishnaiah YS, et al. 2002). Metronidazole, 2-(2-methyl-5-nitro-1H-imidazol-1-yl)-ethanol, is the drug of choice for intestinal amoebiasis and other colon infections (Krishnaiah YS, et al. 2002; Tracy JW, et al. 1996) which is a synthetic antibacterial, anti amoebic and anti protozoal agent of the nitroimidazole class, and it is used against protozoa such as *Trichomonas vaginalis*, Amoebiasis, Giardiasis and extremely effective against anaerobic bacterial infections. It is also used to treat Crohn's disease, antibiotic-associated diarrhoea, and rosachea. The oral bioavailability of Metronidazole is about 100 percent but there are some potential hazards such as peripheral neuropathy and convulsive seizures if the drug is given by conventional dosage form which provides minimal amount for local action in the colon, still resulting in the relief of amoebiasis. The amoeba mainly present in the intra intestinal lumen and the efficient treatment of amoebiasis and other colonic infections could be achieved by targeting drug to the colon and the best approach is a colon targeted specific drug delivery which would make the Metronidazole effective with low dose and prevent the potential hazards.

Overall, there is less free fluid in the colon than in the small intestine (Sarasija, S. and Hota, A. 2000). Hence, dissolution could be problematic for poorly water-soluble drugs. Moreover, the Metronidazole is showing

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good solubility in acidic environment. Therefore, incorporation of organic acids such as Citric acid, Tartaric acid, etc., in the formulation lowers the pH surrounding the system sufficient to effects the dissolution of the drug and enhance the soluble drug available for local and systemic action.

Various approaches available for colon specific drug delivery includes (1) coating with pH dependent systems, (2) design of timed release dosage forms, and (3) the use of carriers that are degraded exclusively by colonic bacteria (Kinget R, et al. 1993; Ramprasad Y V, et al. 1999; V.R. Sinha, et al. 2005; Y. S. R. Krishnaiah, et al. 2002; Evans DF, et al. 1988; Takaya T, et al. 1995; Saffron M, et al. 1986; Wilding IR, et al. 1992). The poor site specificity of pH dependent systems, because of large variation in the pH of the gastrointestinal tract, the timed release systems release their load after a predetermined time period of administration and are designed to resist the release of the drug in the stomach with an additional non disintegrating or lag phase included in the formulation (which equals to the small intestine transit time) and the release of the drug takes place in the colon. The optimistic approach for colon specific drug delivery is the use of carriers that are degraded exclusively by colonic bacteria. Various carriers that are being evaluated for colon specific drug delivery based on the colonic bacterial action are pectin and its salts, Guar gum, etc (Munira Momin, et al. 2008; Munira Momin, 2004). Therefore, the major objective of the present investigation is to prepare matrix tablet using the inexpensive, naturally and abundantly available guar gum and pectin for colon targeted delivery of Metronidazole as the matrix former with the incorporation of organic acids to felicitate the drug solubility in the colon and coating the tablet with suitable polymer for the colon-specific drug delivery.

MATERIALS AND METHODS

Materials

Metronidazole (Mayur chemicals, Chennai- 600003, India), was chosen as model active principle incorporated for the study. Eudragit S 100 was chosen as spray coating material. Guar gum, Pectin, Tartaric acid, Citric acid and Eudragit S 100 were purchased from E. Merck (India) Ltd, Mumbai- 400018, India and Lactose LR, Magnesium stearate (Loba Chem, Mumbai, India) were used as tablet excipients. The other ingredients were used of analytical grade.

Methods

Preformulation study

Preformulation studies emerged in response to the growing interest of the possible stability and compatibility issues of drug formulations. These studies evolved in order to accommodate the urgent need for fast pharmaceutical screening of the increasing number of drug candidates. For the preformulation studies, the drug and the drug with polymer combinations were made. They are mixed together uniformly and placing them for 4 weeks at room temperature. The mixtures were examined every week for their compatibility in terms of texture (morphology and color), spectroscopical study and TLC check.

Fourier Transform Infra Red (FTIR) Analysis

Metronidazole discs were prepared by pressing the drug (2mg) with potassium bromide (200mg) and the spectra between 4000 cm^{-1}- 400 cm^{-1} with a hydrostatic press at a force of 5ι cm^{-2} for 5min and the resolution was 4 cm^{-1} was recorded for the drug raw material and combination with polymers under the operational conditions. Experiments were duplicated to check the reproducibility.

Effect of organic acid on Metronidazole solubility

Excess amount of Metronidazole was transferred into stopper conical flask containing 100 ml of water and six samples were prepared by following the same procedure. Label I was considered as blank and rest of the five (Label II - VI) conical flask added 20, 40, 60, 80 and 100mg of Tartaric acid and the same procedure was followed for Citric acid as well. All the contents were shaken by using rotary mechanical shaker for 6h at 120rpm. The withdrawn solution was filtered through Whatman filter paper. A 5ml of filtrate from the flasks was taken and diluted further (1ml to 100ml and 1ml to 10ml for Tartaric acid and 1ml to 100ml for Metronidazole). The drug concentration was analyzed using UV spectrophotometer at λ_{max} 276 nm.

Formulation of matrix tablets

Matrix tablets were prepared by wet granulation technique. Accurately weighed quantity of all ingredients were passed through sieve # 60 and the quantities were blended uniformly for 10 min. Starch (10%) was used as a binder and distilled water was used as a solvent for mucilage preparation. The aggregates were initially dried for 5-10 minutes and then passed through sieve # 20 to get granules. The granules were dried at 50ºC for 60 min to reduce moisture content to 2-5%. the prepared granules were lubricated with Talc and Magnesium Stearate (2:1 ratio). Same method was followed for all formulations and the formulas for all the formulations containing guar gum and pectin in different concentrations with citric acid and tartaric acid are given in Table 1 and Table 2 respectively. The granules were evaluated for flow property, bulk density and compressibility of all the formulations and they were punched in to tablets of average weight 800mg using 8 station rotary tablet punching machine. The formulated matrix tablets were evaluated for their physicochemical evaluations.

Compression coating of matrix tablets

The formulated matrix tablets were taken for further compression coating and 200 mg of compression coating material (Eutragit S 100) was applied over the core

Table 1: Formulas for the formulations of Matrix tablets containing guar gum and pectin in different concentrations with citric acid

S.No	Ingredients	Quantity per Tablet in mg					
		MGC F1	MGC F 2	MGC F3	MPC F4	MPC F5	MPC F6
1.	Metronidazole	400	400	400	400	400	400
2.	Guar gum	120	160	200	-	-	-
3.	Pectin	-	-	-	120	160	200
4.	MCC	72	52	32	72	52	32
5.	Starch	72	52	32	72	52	32
6.	Citric acid	80	80	80	80	80	80
7.	Talc	10	10	10	10	10	10
8.	Magnesium stearate	6	6	6	6	6	6
9.	Starch Paste (10%)	40	40	40	40	40	40

Table 2: Formula for the formulations of Matrix tablets containing guar gum and pectin in different concentrations with tartaric acid

S.No	Ingredients	Quantity per Tablet in mg					
		MGT F7	MGT F 8	MGT F9	MPT F10	MPT F11	MPT F12
1.	Metronidazole	400	400	400	400	400	400
2.	Guar gum	120	160	200	-	-	-
3.	Pectin	-	-	-	120	160	200
4.	MCC	72	52	32	72	52	32
5.	Starch	72	52	32	72	52	32
6.	Tartaric acid	80	80	80	80	80	80
7.	Talc	10	10	10	10	10	10
8.	Magnesium stearate	6	6	6	6	6	6
9.	Starch Paste (10%)	40	40	40	40	40	40

Table 3: Granule evaluation of all the formulations of Matrix tablets containing guar gum and pectin in different concentrations with citric acid and tartaric acid

S.NO	Formulations	Angle of Repose*	Bulk Density*	Corr's Index*
1	MGC 1	29.36°	0.524	16.52
2	MGC 2	27.43°	0.497	14.92
3	MGC 3	26.98°	0.481	13.62
4	MPC 4	29.76°	0.510	15.51
5	MPC 5	26.12°	0.449	10.53
6	MPC 6	26.56°	0.442	12.42
7	MGT 7	27.66°	0.498	16.32
8	MGT 8	26.53°	0.448	12.12
9	MGT 9	27.91°	0.483	15.92
10	MPT 10	25.66°	0.421	11.56
11	MPT 11	27.41°	0.479	14.53
12	MPT 12	29.59°	0.492	16.46

Limits: Angle of Repose ≈ 25-30° indicates free flowing material. Carr's index 5-15% indicates free flowing material. * Results are replicates of three.

tablets. For compression coating, 25% of coat weight was placed in the die cavity followed by carefully centering the core tablet and addition of the remainder of coat weight. The coating material was compressed around the core tablet at an applied force of 5000 kg using 10 mm round concave punches using 8-station rotary tablet machine and evaluated for their physicochemical evaluations. The results are shown in Table nos: 6-8.

Spray coating of matrix tablets

The formulated matrix tablets were taken for further spray coating with Eutragit S 100 coating solution.

i. Preparation of Eudragit S100 dispersion:

The solution of Eudragit S100 was prepared by dissolving 10gm of Eudragit S100 dry powder in the mixture of 65ml of isopropyl alcohol (IPA) and 5ml of water at room temperature.

Table 4: Physical evaluation of the matrix formulations of Matrix tablets containing guar gum and pectin in different concentrations with citric acid and tartaric acid

S.NO	Formulations	Thickness* (mm)	Diameter* (cm)	Hardness* (kg/cm^2)
1	MGC 1	3.9 ± 0.02	1.1 ± 0.02	3.8 ± 0.12
2	MGC 2	3.9 ± 0.01	1.2 ± 0.11	4.0 ± 0.14
3	MGC 3	4.0 ± 0.02	1.1 ± 0.03	4.2 ± 0.17
4	MPC 4	4.1 ± 0.02	1.1 ± 0.01	4.2 ± 0.12
5	MPC 5	4.1 ± 0.01	1.2 ± 0.09	4.0 ± 0.21
6	MPC 6	3.9 ± 0.03	1.1 ± 0.13	4.2 ± 0.08
7	MGT 7	3.8 ± 0.01	1.0 ± 0.03	4.1 ± 0.16
8	MGT 8	4.0 ± 0.02	1.2 ± 0.10	3.9 ± 0.12
9	MGT 9	4.1 ± 0.02	1.2 ± 0.02	4.0 ± 0.14
10	MPT 10	4.2 ± 0.03	1.2 ± 0.02	4.2 ± 0.10
11	MPT 11	4.2 ± 0.02	1.1 ± 0.02	4.0 ± 0.18
12	MPT 12	3.8 ± 0.02	1.1 ± 0.03	4.1 ± 0.13

Table 5: Assay and swelling and abrasion evaluation of the formulations of Matrix tablets with guar gum and pectin in different concentrations with citric acid and tartaric acid

S.NO	Formulations	Drug Content* (%)	Friability* (%)	Swelling index* (%)
1	MGC F1	101.29	0.18	112
2	MGC F2	98.72	0.34	119
3	MGC F3	99.14	0.42	127
4	MPC F4	100.05	0.29	102
5	MPC F5	99.76	0.46	107
6	MPC F6	102.32	0.53	111
7	MGT F7	101.91	0.29	115
8	MGT F8	99.46	0.54	120
9	MGT F9	102.91	0.32	130
10	MPT F10	101.27	0.26	105
11	MPT F11	99.81	0.34	109
12	MPT F12	98.98	0.63	110

* Results are replicates of three.

Table 6: Formula for the formulations of Compression coated tablets containing guar gum and pectin in different concentrations with Citric acid

S.No	Ingredients	Quantity per Tablet in mg					
		MGCE F13	MGCE F 14	MGCE F15	MPCE F16	MPCE F17	MPCE F18
1.	Metronidazole	400	400	400	400	400	400
2.	Guar gum	120	160	200	-	-	-
3.	Pectin	-	-	-	120	160	200
4.	MCC	72	52	32	72	52	32
5.	Starch	72	52	32	72	52	32
6.	Citric acid	80	80	80	80	80	80
7.	Talc (%)	10	10	10	10	10	10
8.	Magnesium stearate (%)	6	6	6	6	6	6
9.	Starch Paste (10%)	40	40	40	40	40	40
10.	Eudragit S 100 (Compression coated)	200	200	200	200	200	200

ii. Preparation of Eudragit S100 organic coating solution:

In the 75ml of IPA 2.0 gm of tannic and 1.0ml of PEG 400 were added with constant stirring after a homogenous mixture was obtained, the 70 ml of Eudragit S100 dispersion was added with a continuous stirring.

Table 7: Formula for the formulations of Compression coated tablets containing guar gum and pectin in different concentrations with tartaric acid

S.No	Ingredients	Quantity per Tablet in mg					
		MGTE F19	MGT E F 20	MGTE F21	MPTE F22	MPTE F23	MPTE F24
1.	Metronidazole	400	400	400	400	400	400
2.	Guar gum	120	160	200	-	-	-
3.	Pectin	-	-	-	120	160	200
4.	MCC	72	52	32	72	52	32
5.	Starch	72	52	32	72	52	32
6.	Tartaric acid	80	80	80	80	80	80
7.	Talc (%)	10	10	10	10	10	10
8.	Magnesium stearate (%)	6	6	6	6	6	6
9.	Starch Paste (10%)	40	40	40	40	40	40
10.	Eudragit S 100 (Compression coated)	200	200	200	200	200	200

Table 8: Physical evaluation for the formulations of Compression coated tablets containing guar gum and pectin in different concentrations with citric acid and tartaric acid

S.NO	Formulations	Thickness mm	Diameter cm	Hardness Kg/cm^2	Weight(mg) n=20
1	MGCE F13	3.5± 0.01	1.6 ± 0.09	5.3 ± 0.10	998±2.5
2	MGCE F14	3.6± 0.04	1.7± 0.12	5.2 ± 0.11	1016±1.8
3	MGCE F15	3.5± 0.03	1.6± 0.07	5.6 ± 0.14	996±2.2
4	MPCE F16	3.5± 0.02	1.6 ± 0.10	4.8 ± 0.09	997±2.4
5	MPCE F17	3.6± 0.03	1.6 ± 0.06	4.9 ± 0.16	1018±1.7
6	MPCE F18	3.5± 0.02	1.6 ± 0.10	4.8 ± 0.09	1021±2.4
7	MGTE F19	3.5± 0.02	1.6± 0.02	5.2 ± 0.12	1014±1.8
8	MGTE F20	3.6± 0.03	1.7 ± 0.04	5,4 ± 0.13	1023±0.8
9	MGTE F21	3.5± 0.01	1.6± 0.02	5.0 ± 0.17	994±1.4
10	MPTE F22	3.5± 0.01	1.6 ± 0.01	4.8± 0.11	1016±1.2
11	MPTE F23	3.6± 0.03	1.7 ± 0.03	4.7 ± 0.06	1021±0,9
12	MPTE F24	3.5± 0.02	1.6 ± 0.01	4.7 ± 0.09	996±1.5

Table 9: Formula for the formulations of Spray coated tablets containing guar gum and pectin in different concentrations with Citric acid

S.No	Ingredients	Quantity per Tablet in mg					
		MGCES F25	MGCES F 26	MGCES F27	MPCES F28	MPCES F29	MPCES F30
1.	Metronidazole	400	400	400	400	400	400
2.	Guar gum	120	160	200	-	-	-
3.	Pectin	-	-	-	120	160	200
4.	MCC	72	52	32	72	52	32
5.	Starch	72	52	32	72	52	32
6.	Citric acid	80	80	80	80	80	80
7.	Talc (%)	10	10	10	10	10	10
8.	Magnesium stearate (%)	6	6	6	6	6	6
9.	Starch Paste (10%)	40	40	40	40	40	40
10.	Eudragit S 100 (Spray coated)	80	80	80	80	80	80

iii. Coating of Metronidazole Matrix tablets using Eudragit S100 coating solution:

The coating dispersion were passed through sieve 0.25mm aperture diameter and The spray rate was set to be 2ml/min at the compression air pressure was maintained at 12psi for better spray of polymer solution. The polymer solution was sprayed at constant rate (2ml/min.) over the bed which was rotated at 20 rpm and controlled temperature (40 ± 2°C).Before coating the tablets were preheated to 40^{0}C for 15 min. The spray was continued until 10%w/w (weight gain was 80mg/Tablet) of total weight gain. The results are shown in Table nos: 9-11.

Table 10: Formula for the formulations of Spray coated tablets containing guar gum and pectin in different concentrations with Tartaric acid

S.No	Ingredients	Quantity per Tablet in mg					
		MGTES F31	MGTES F 32	MGTES F33	MPTES F34	MPTES F35	MPTES F36
1.	Metronidazole	400	400	400	400	400	400
2.	Guar gum	120	160	200	-	-	-
3.	Pectin	-	-	-	120	160	200
4.	MCC	72	52	32	72	52	32
5.	Starch	72	52	32	72	52	32
6.	Tartaric acid	80	80	80	80	80	80
7.	Talc (%)	10	10	10	10	10	10
8.	Magnesium stearate (%)	6	6	6	6	6	6
9.	Starch Paste (10%)	40	40	40	40	40	40
10.	Eudragit S 100 (Spray coated)	80	80	80	80	80	80

Table 11: Physical evaluation for the formulations of Spray coated tablets containing guar gum and pectin in different concentrations with citric acid and tartaric acid

S.NO	Formulations	Thickness mm	Diameter cm	Hardness Kg/cm^2	Weight(mg) n=20
1	MGCES F25	3.3± 0.04	1.4± 0.08	5.6 ± 0.10	880±1.5
2	MGCES F26	3.4± 0.01	1.5± 0.12	5.4 ± 0.11	870±2.2
3	MGCES F27	3.3± 0.03	1.4± 0.09	5.6± 0.12	884±1.8
4	MPCES F28	3.3± 0.07	1.4 ± 0.07	4.9 ± 0.14	886±1.7
5	MPCES F29	3.4± 0.01	1.5± 0.12	4.8 ± 0.16	875±2.1
6	MPCES F30	3.3± 0.05	1.4 ± 0.16	4.9 ± 0.09	872±1.8
7	MGTES F31	3.3± 0.04	1.4± 0.14	5.4 ± 0.13	882±1.7
8	MGTES F32	3.3± 0.02	1.5± 0.06	5.3 ± 0.15	884±1.4
9	MGTES F33	3.3± 0.01	1.4± 0.13	5.4 ± 0.13	892±1.3
10	MPTES F34	3.3± 0.01	1.5 ± 0.07	4.7 ± 0.14	877±1.8
11	MPTES F35	3.4± 0.01	1.4± 0.16	4.9 ± 0.13	890±1.7
12	MPTES F36	3.3± 0.02	1.4± 0.15	5.0 ± 0.16	892±0.9

Swelling studies on matrix tablets

The swelling index of the tablets was performed to comprehend the influence of swelling and erosion behavior of the formulation on its drug release. In this study, tablets were weighed accurately (W_0) and placed in Petri dish containing 10 ml of distilled water. At the end of 2 hours, the tablets were removed from the Petri dish and the excess surface water was removed carefully using filter paper and swollen tablets were reweighed (W_t). The swelling index was calculated according to the formula (1);

$$\text{Swelling Index} = \frac{(W_t - W_0)}{W_0} \times 100 \longrightarrow (1)$$

Where,

W_t is the weight of Tablet at time 't'.

W_0 is the weight of Tablet at time t = 0.

Drug Content

Ten tablets of each formulation were weighed and powdered. The quantity of powder equivalent to 400 mg of metronidazole and Tinidazole was transferred in to a 100 ml volumetric flask and extracted with 0.1N hydrochloric acid and kept aside for 2 hours. Then it was filtered, suitable dilutions were made and absorbance was measured by using SHIMADZU UV- Visible spectrophotometer at 276 nm and 282 nm respectively. The results listed in Table no: 5.

***In vitro* release studies**

As the preparation is for to release the drug in the colon through oral administration, three different receptor fluids are used for evaluation of the dissolution profile.

i. Acid stage

900 ml of 0.1N hydrochloric acid was placed in vessel and the USP standard dissolution apparatus -II (paddle method) was assembled. The medium was allowed to equilibrate to temperature of 37 ± 1°C. tablet was placed in the vessel and the apparatus was operated for 2 hours at 50 rpm as the mean gastric transit time is two hours. At each one hour interval 10 ml of receptor fluid was withdrawn, filtered, suitable dilutions were done with 0.1N hydrochloric acid.

ii. pH 7.4 phosphate buffer

Tablets are removed from the above acid medium after 2 hours and the medium was replaced with 900 ml of buffer pH 7.4 solution and the dissolution continued for 3 hours (2-5 hours) as the mean intestinal transit time is 3 hours, and the sample volume of 10 ml with drawn at different intervals, filtered and suitable dilutions were made with pH 7.4 phosphate buffer.

iii. pH 6.8 phosphate buffer

To the above medium 90 ml of 0.1 N hydrochloric acid was added and the pH adjusted to 6.8 and volume made up to 1000 ml. the dissolution continued up to 24 hours as the mean colon transit time is 24 hours and maintaining the same conditions and the sample volume of 10 ml with drawn at different intervals, filtered and suitable dilutions were made with pH 7.4 phosphate buffer. All the samples were analyzed spectrophotometrically at 276nm.

***In vitro* dissolution study of best formulations in Rat cecal content (4%w/v):**

Total six number of male Wister rats weighing 150-250 gm were maintained on room temperature ranging 28-30°C. The normal diet (standard rat diet) was given along with water. The procedure involved oral treatment of rats with 1 ml of 1% w/v guar gum and pectin dispersion for 7 days. The rat cecal content medium at 4% w/v level obtained after 7 days of enzyme induction with 1 ml of 1% w/v guar gum and pectin dispersion provide the best conditions for assessing the susceptibility of guar gum and pectin to colonic bacterial degradation. The rats were sacrificed just thirty minutes before the commencement of drug release studies, the rats abdomen was opened, ligatures were made before and after the cecum and the cecum was removed and cecum bag was opened and its content were weighed and homogenized, then suspended in PBS (pH 6.8) to give the concentration of 4% w/v of cecal content. The *In vitro* studies were performed in almost anaerobic conditions (in CO2 atmosphere). Samples were withdrawn and replaced with fresh dissolution media. The studies were performed for 12 hours.

RESULT AND DISCUSSION

The drug and polymer interaction studies showed that there is no change in their physicochemical property during these 4 weeks. Hence, the polymers tested could be taken for further studies.

In FTIR study, Metronidazole showed some characteristic bands between 4000 – 400 cm^{-1} range like the main peaks between 3100 –2990, 1680 – 1650, 1667 – 1653, 1354 – 1268, and 864 – 825, which identifies the presence of C-H Stretching, C=C Stretching, C-N Stretching, N=O Stretching and N-O Stretching respectively. The same peaks were identified in the drug blended with polymers (Guar gum, Pectin and other additives) such main peaks as between 3500 – 3200, 3100 –2990, 1680 – 1650, 1667 – 1653, 1354 – 1268, and 864 – 825, which identifies the presence of free –OH group in the polymers, C-H Stretching, C=C Stretching, C-N Stretching, N=O Stretching and N-O Stretching as in the drug raw material respectively.

The FTIR spectra of the physical mixture of the drug with polymers exhibited all the characteristic bands as in the spectrum of the individual Metronidazole, Guar gum, Pectin and other excipients excluding the possibility of any interaction, chemical and functional group change during the processing of the formulation of matrix tablets is ensured.

In the effect of organic acid on the solubility of Metronidazole, gradual increase of drug solubility was observed as the acid concentration increases up to 80mg but drastic change in the solubility was observed when the concentration increased more than 80mg in the study. In terms of pH, 80mg of tartaric acid containing solution was showing about 3.4, which would definitely increase the solubility of the drug in colon. Therefore, it was decided to use 80mg of organic acid in all the formulations to improve the solubility of the drug when the exposures in colon where very less amount of aqueous fluid is available. The effect of organic acid on Metronidazole solubility in water is shown in Figure 1.

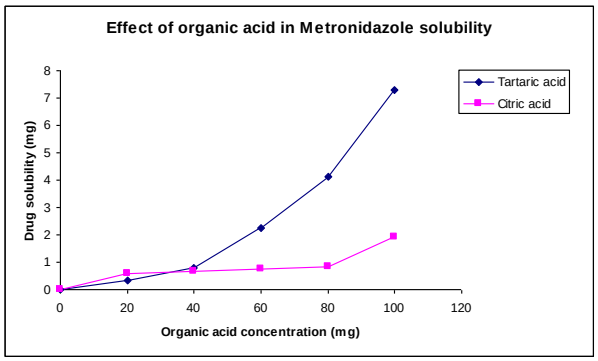

Figure 1: Effect of organic acid on Metronidazole solubility in water

Granules were prepared by wet granulation technique and the prepared granules were evaluated for their suitability of matrix formulations. Bulk density ranged between 0.439 – 0.512 which showed good packing characters, angle of repose ranged between 25°-30° which showed good flow property and the Carr's index ranged between 11 – 17 indicated free flowing materials suitable for best packing provides uniformity of drug in formulations of all formulations. The results obtained on granule evaluation are shown in Table 3.

Matrix tablets were formulated using guar gum and pectin in different proportions along with organic acid using 8 station tablet compression machine. Three formulations of Metronidazole with guar gum and citric acid (MGC F1 to F3), three formulations of Metronidazole with pectin and citric acid (MPC F4 to F6), three formulations of Metronidazole with guar gum and tar-

taric acid (MGT F7 to F9), three formulations of Metronidazole with pectin and tartaric acid (MPT F10 to F12) of total 12 formulations as matrix tablets were formulated by wet granulation technique. The matrix formulations were taken for compression coating with Eudragit RS 100 (17.5%), and the formulations are coded as (MGCE F13 to F15), (MPCE F16 to F18), (MGTE F19 to F21), and (MPTE F21 to F24). The matrix formulations were taken for further spray coating with Kollicoat MAE 30 DP (5%), and the formulations are coded as (MGCES F25 to F27), (MPCES F28 to F30), (MGTES F31 to F33), and (MPTES F34 to F36).

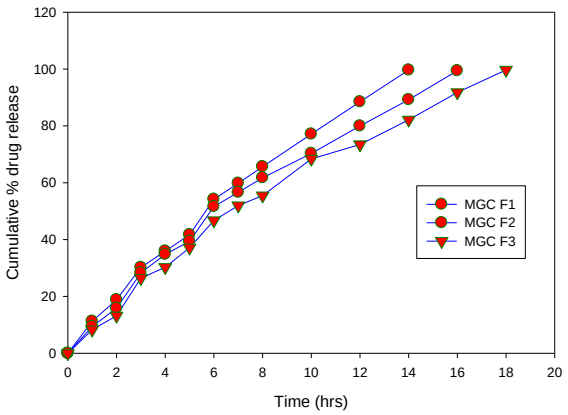

Figure 2: *In vitro* drug release for the formulations of Metronidazole Matrix tablets containing guar gum in different concentrations with citric acid

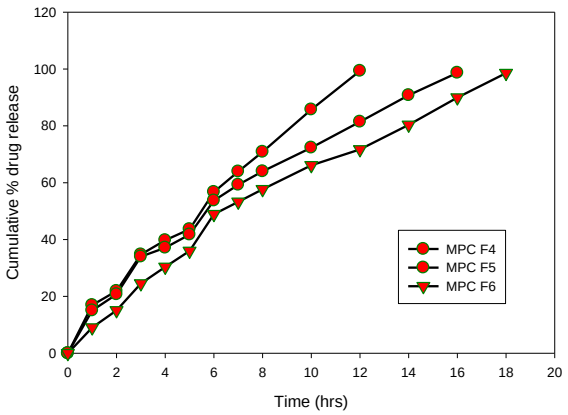

Figure 3: *In vitro* drug release for the formulations of Metronidazole Matrix tablets containing pectin in different concentrations with citric acid

All the formulations were evaluated their physical properties such as Thickness (mm), Hardness (kg/cm^2) and Diameter (cm) and the results are shown in Table 4. Thickness of all the formulations ranged between 3.5 – 4.5mm, diameter of repose for all formulations ranged between 1–1.5cm and the hardness of all formulations ranged between 3 – 5 kg/cm^2. All the formulations were also evaluated their properties such as drug content, Friability and Swelling index and the results are shown in Table 5. Friability (%) of all the formulations ranged between 0.1 – 0.7, drug content (%) of all formulations ranged between 98 - 103 and the swelling index (%) of all formulations ranged between 100 - 135. All the physicochemical evaluations of all the batches were with the limit which indicated the suitability of the formulation technique. In swelling study, all the formulations taken almost equal weight of water and the formulation contain Gur gum showed more than the equal weight of water ensures enough swelling to solubilize the drug.

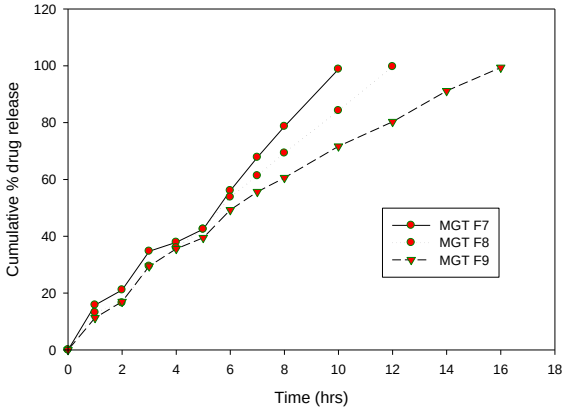

Figure 4: *In vitro* drug release for the formulations of Metronidazole Matrix tablets containing guar gum in different concentrations with Tartaric acid

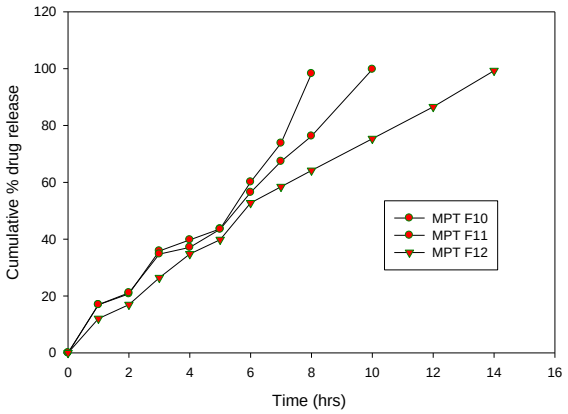

Figure 5: *In vitro* drug release for the formulations of Metronidazole Matrix tablets containing pectin in different concentrations with Tartaric acid

The dissolution profiles of tablets were determined by using dissolution apparatus (USP XXII apparatus) taking buffer solutions of 0.1N hydrochloric acid (2 h), pH 7.4 (3 h), and pH 6.8 (7 h). The matrix formulations, MGC F1 to F3 and MGT F7 to F9 showed maximum drug release of about 21% but MPC F4 to F6 and MPT F10 to F12 showed fast release and maximum of 25% in acidic pH at 2hr and MGC F1 to F3 and MGT F7 to F9 showed maximum drug release of about 43% but MPC F4 to F6 and MPT F10 to F12 showed fast release and maximum of 50% in phosphate buffer pH 7.4 at 5hr. Release

study in phosphate buffer pH 6.8, formulation containing low concentration of pectin(30%) was completely released the drug with in 12hr but low concentration of guar gum(30%) somewhat controlled the drug release but not extended till 12 hr. At mid concentrations, guar gum containing formulation showed the drug release in controlled manner but high concentrations, the drug release was not complete at 12th hr.

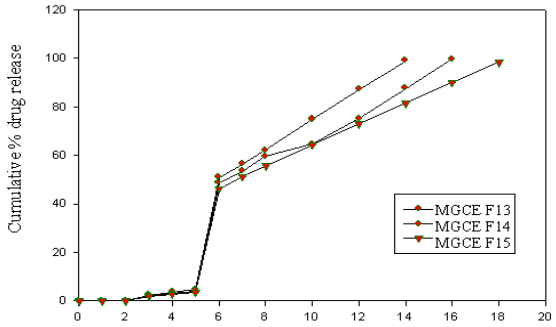

Figure 6: *In vitro* drug release for the formulations of Metronidazole Compression coated tablets containing guar gum in different concentrations with citric acid

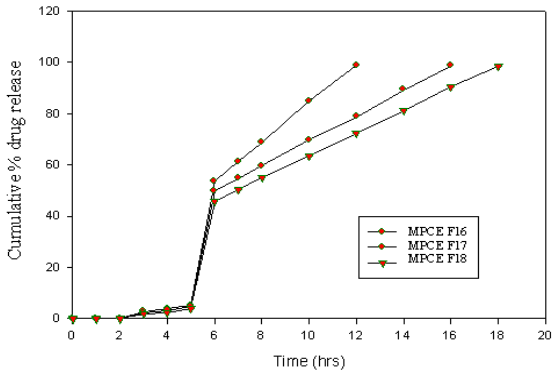

Figure 7: *In vitro* drug release for the formulations of Metronidazole Compression coated tablets containing pectin in different concentrations with citric acid

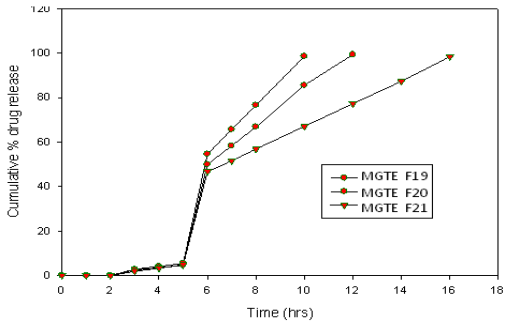

Figure 8: *In vitro* drug release for the formulations of Metronidazole Compression coated tablets containing guar gum in different concentrations with Tartaric acid

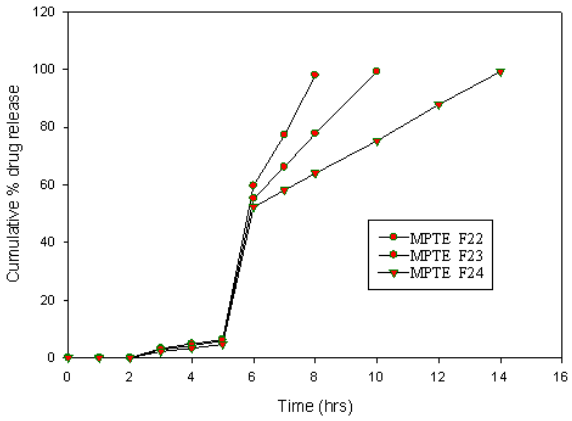

Figure 9: *In vitro* drug release for the formulations of Metronidazole Compression coated tablets containing pectin in different concentrations with Tartaric acid

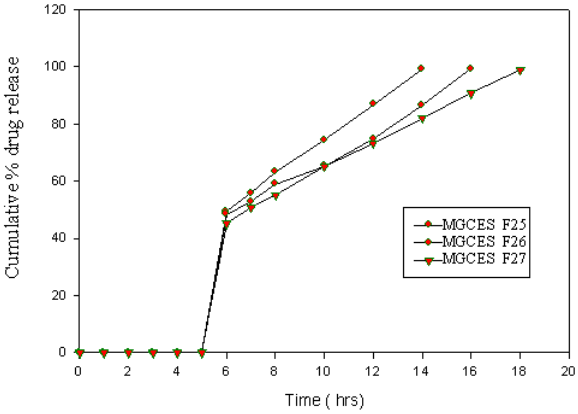

Figure 10: *In vitro* drug release for the formulations of Metronidazole Spray coated tablets containing guar gum in different concentrations with Citric acid

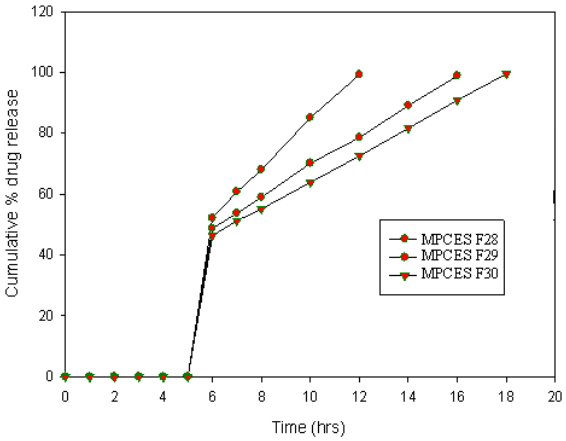

Figure 11: *In vitro* drug release for the formulations of Metronidazole Spray coated tablets containing pectin in different concentrations with Citric acid

The compression coated formulations, no drug release was observed in acidic pH till 2hr, and formulations MGCE F13 to F15 and MGTE F19 to F21 showed maximum drug release of about 5% but MPCE F16 to F18, MPTE F22 to F24, showed fast release and maximum of 7% in phosphate buffer pH 7.4 at 5hr. Release study in

phosphate buffer pH 6.8, formulation containing low concentration of pectin(30%) was completely released the drug with in 10hr but low concentration of guar gum(30%) somewhat controlled the drug release but not extended till 12 hr. At mid concentrations, guar gum with Tartaric acid containing formulation showed the drug release in controlled manner but high concentrations, the drug release was not complete at 12th hr.

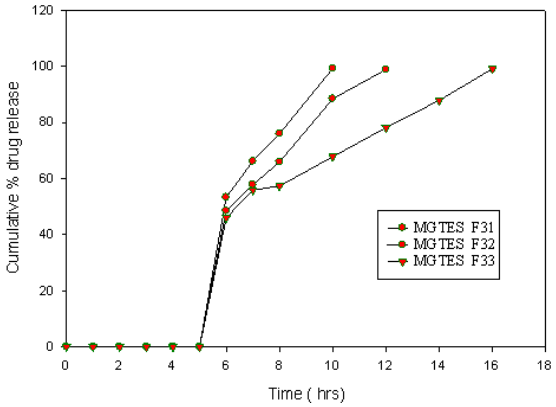

Figure 12: *In vitro* drug release for the formulations of Metronidazole Spray coated tablets containing guar gum in different concentrations with Tartaric acid

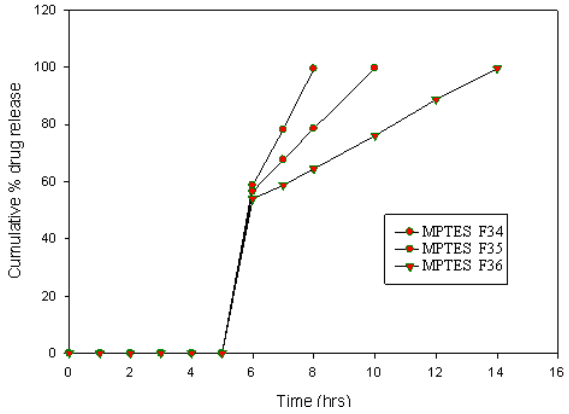

Figure 13: *In vitro* drug release for the formulations of Metronidazole Spray coated tablets containing pectin in different concentrations with Tartaric acid

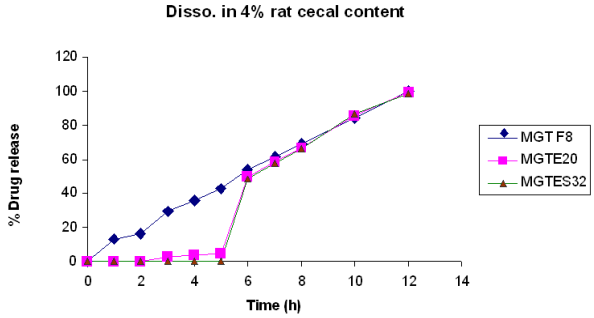

Figure 14: The release profile of the best formulations in rat cecal content (4%)

The spray coated formulations, no drug release was observed in acidic pH till 2hr, in phosphate buffer pH 7.4, and guar gum containing spray coated formulations, MGCK F25 to F27 and MGTK F31 to F33 showed controlled drug release completely at 12hr in low and medium concentration, and high concentration the complete drug release extended up to 20 hr but pectin containing spray coated formulations, MPCK F28 to F30 and MPTK F34 to F36 showed fast release at low concentration, controlled release at medium concentration and at high concentration, the complete release was extended up to 18 hr in phosphate buffer pH 7.4 at 5hr.

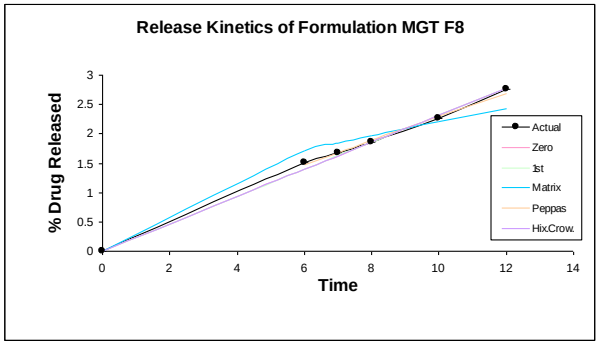

Figure 15: The release kinetics of the best formulation MGT F8

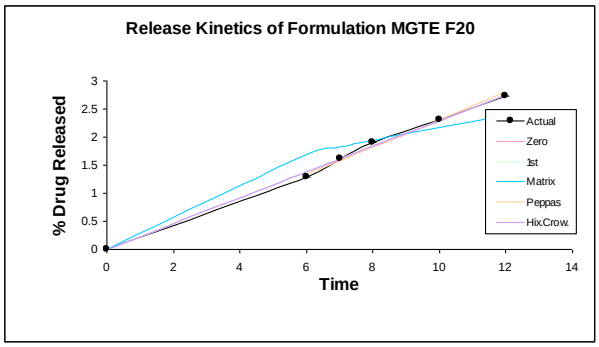

Figure 16: The release kinetics of the best formulation MGTE F20

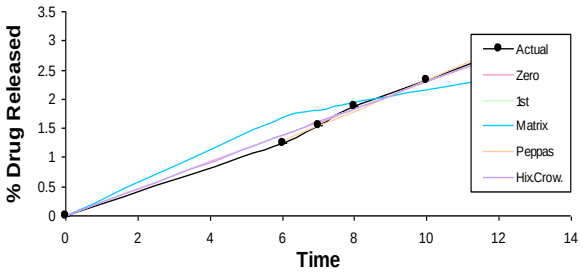

Figure 17: The release kinetics of the best formulation MGTES F32

At the same time, all of the formulations with Tartaric acid showed more drug release than the formulation containing citric acid. Release study in phosphate buffer pH 6.8, formulation containing low concentration of pectin (30%) was completely released the drug with in 10hr but low and medium concentration of Guar

gum(30% and 40%) controlled the drug release. At medium concentration of Guar gum with Tartaric acid containing formulation showed the drug release in controlled manner.

The best formulations (MGT F8, MGTE F20, and MGTK F32) were selected based on the percentage of drug release in the targeted time period, and the selected formulations were allowed for dissolution study in rat cecal content (4% w/v) suspended in 6.8 pH phosphate buffer. The release profile of the best formulations is shown in Figure 14.

The drug release of the formulations were analyzed by Zero order, first order, Peppas, Higuchi, Kornsmayer peppas ('n' value) to determine the mechanism of drug release using PCP-Disso Excel Software. The best fit model of formulations MGT F8, MGTE F20, and MGTK F32 were found to be first order (r^2 -0.9981, n value - 0.8734), zero order (r^2 - 0.9986, n - 1.0601), and zero order (r^2 -0.9974, n - 1.1501) respectively. The release kinetics of the best formulations MGT F8, MGTE F20, and MGTK F32 is shown in Figure 15, Figure 16, and Figure 17 respectively.

CONCLUSION

Successful colonic delivery requires careful consideration of a number of factors, including the properties of the drug, the type of delivery system and its interaction with the healthy or diseased gut. Although a number of formulations have been proposed as colonic delivery vehicles, most lack the necessary of site specificity. The only universal system currently marketed is pH dependent systems, but this has shown pre-release of the drug in GI tract. Matrix tablets belong to oral drug delivery system that are capable of releasing the drug in the colon with optimum result was formulated and they were further coated successfully by compression and spray coating techniques to optimistically target drug release to the colon without premature drug release. Guar gum at 40% was found to be best in controlled release along with Tartaric acid (80mg) which may help to enhance the drug solubility in colon where minimal aqueous environment is present. Preformulation studies confirmed that there was no drug to polymer interaction, all the physicochemical studies performed on drug, granules and the formulations were falling within the limit and shown feasibility in their formulations. From the result obtained on drug release study of the spray coated formulations found to be suitable for colon-specific drug delivery. The guar gum matrix tablets showed a slightly slower rate of drug release and slightly high residence time when compared with pectin matrix tablets. The guar gum compression coated tablets and spray coated tablets are showed a slightly slower rate of drug release and slightly high residence time when compared with pectin compression and spray coated tablets. In term of release kinetic studies, the best formulations i.e., MGTE F20 and MGTK F32 showed the desired zero order drug release. Hence, this special drug delivery system is needed to minimize the hazardous effects and to increase the effectiveness of the drug and there is a need of to investigate a number of indigenously available retardant materials to make the concept of colon specific drug delivery more viable for the industry at more economical way.

REFERENCES

Evans DF, Pye G, Bramley R, Hardcastle JD. Gut 1988, 29:1035-1041.

Haque R, Mondal D, Duggal P, *et al.* (2006). "*Entamoeba histolytica* infection in children and protection from subsequent amebiasis". *Infection & Immunity* 74 (2): 904–909.

Kinget R, Van-Den-Mooter G, Samyn C. Azopolymers for colon-specific drug delivery, Part 2. Influence of the type of azopolymer on degradation by intestinal microflora, International journal of Pharmaceutics, 93, 1993, 133-139.

Kinget R., Kalala W., Vervoort L. and van den Mooter G. Colonic drug delivery. J. Drug Target.1998,6,129-149.

Krishnaiah YS, Bhaskar reddy PR, Satyanarayana S, Karthikeyan RS. Studies on the development of oral colon targeted drug delivery systems for metronidazole in the treatment of amoebiasis. Int J Pharmaceu. 2002; 236(1-2): 43-55.

Mondal D, Petri Jr WA, Sack RB, *et al.* (2006). "*Entamoeba histolytica*-associated diarreal illness is negatively associated with the growth of preschool shildren: evidence from a prospective study". *Trans R Soc Trop Med H* 100 (11): 1032–38.

Munira Momin, K. Pundarikakshudu, and S. A. Nagori, Design and Development of Mixed Film of Pectin: Ethyl Cellulose for Colon Specific Drug Delivery of Sennosides and *Triphala,* Indian J Pharm Sci. 2008 May–Jun; 70(3): 338–343.

Munira Momin, K. Pundarikakshudu, In vitro studies on guar gum based formulation for the colon targeted delivery of sennosides, Pharm Pharmaceut Sci 7(3):325-331, 2004.

Ramprasad Y V, Krishnaiah Y S, Satyanarayana S. Studies of guar gum compression-coated 5-aminosalicylic acid tablets for colon-specific drug delivery, Drug development and industrial pharmacy, 25(5), 1999, 651-657.

Saffron M, Kumar GS, Sabvariar C, Burnham JC, Williams F, Neckers DC. Science 1986, 233: 1081-1084.

Sarasija, S. and Hota, A., Colon-specific drug delivry systems, Ind J Pharm Sci, 62: 1-8, 2000.

Takaya T, Ikada C, Imagawa N, Niwa K, Takada K. J Pharm Pharmacol 1995, 47:474-478.

Tracy JW, Webster LT Jr. (1996). Drugs used in the Chemotherapy of protozoal infections. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition, (Ed) Aardman JG, Goodman A, Gilman L, Limbird E, McGraw H USA pp. 1069-1086.

V.R. Sinha, B.R. Mittal and Rachna Kumria, In vivo evaluation of time and site of disintegration of polysaccharide tablet prepared for colon-specific drug delivery, International Journal of Pharmaceutics, 289 (1-2), 2005, 79-85.

WHO. "*Amoebiasis. Report of a WHO Expert Committee".* WHO Technical Report Series, 1969, *421: 1–52.*

WHO. "*WHO/PAHO/UNESCO report. A consultation with experts on amoebiasis, Mexico City, Mexico,* Epidemiological Bulletin, 1997, *18 (1): 13–14.*

Wilding IR, Davis SS, Bakhshaee M, Stevens HNE, Sparrow RA, Brennan J. Pharm Res 1992, 9: 654-657.

Y. S. R. Krishnaiah, V. Satyanarayana, B. Dinesh Kumar and R. S. Karthikeyan, In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil, European Journal of Pharmaceutical Sciences, Volume 16, Issue 3, August 2002, Pages 185-192.