Colon Targeted Delivery Systems of Metronidazole Based on Osmotic Technology: Development and Evaluation

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Colon targeted delivery systems of metronidazole (MTZ) based on osmotic technology were developed. The developed systems consisted of osmotic core (drug, osmotic agent and wicking agent), coated with semipermeable membrane (SPM) containing guar gum as pore former, coated core were then further coated with enteric coating to protect the system from acidic environment of stomach. The effect of various formulation variables namely the level of wicking agent (sodium lauryl sulphate), osmotic agent in the osmotic core, the level of pore former (guar gum) in SPM, and the thickness of SPM, were studied on physical parameters and drug release characteristics of developed formulations. MTZ release was inversely proportional to SPM thickness, but directly related to the level of pore former, wicking agent and osmotic agent. On the other hand burst strength of the exhausted shells was decreased with the increase in level of pore former in the membrane but increased with the increase in the thickness of SPM. The drug release from the developed formulations was independent of pH, and agitation intensity, but dependent on the osmotic pressure of the release media. The thickness of enteric coating could prevent formation of delivery pores before contact with simulated colonic fluid, but had no effect on drug release. Result of SEM studies showed the formation of *in-situ* delivery pores in the membrane from where the drug release occurred, and the number of pores formed were directly related to the initial level of pore former (guar gum) in SPM. The manufacturing procedure was found to be reproducible and formulations were found to be stable during 3 months of accelerated stability studies.

Key words colon targeted; metronidazole; semipermeable membrane; guar gum; osmotic technology; stability

Metronidazole (MTZ) is the most preferred choice of drugs for intestinal amoebiasis.[1] This drug is to be delivered to the colon for its effective action against *Entamoebe histolytica* wherein the trophozoites reside in the lumen of the caecum and large intestine and also adhere to the colonic mucus and epithelial layers.[2] But the pharmacokinetic profile of metronidazole indicates that the drug is completely and promptly absorbed after oral administration and plasma concentration of about 10 μg/ml is reached approximately 1 h after a single 500 mg dose.[3] The administration of this drug in conventional tablet dosage form provides minimal amount of metronidazole for local action in the colon, still resulting in the relief of amoebiasis, but with unwanted systemic effects. Thus there is strong clinical need and market potential for a delivery system that will deliver maximum amount of MTZ to the colon in controlled manner.

Colon targeted delivery systems are well recognized and documented to deliver most of the drugs to colon. In the past, various primary approaches for colon targeted delivery, such as, prodrugs approach,[4] pH,[5] and time[6] and pressure dependent systems,[7] have achieved limited success. The majority of these systems, developed during the past decade, were based on pH and time dependent mechanisms with limited *in-vivo* evaluation.[8] Minor variation in pH between the small intestine and the colon makes the pH-dependent systems less specific, in terms of targeted release in the colon. Time-dependent systems predominantly depend on the transit time of the delivery system in the gastrointestinal tract (GIT). A major limitation with these systems is that *in vivo* variation of the small intestinal transit time may lead to release of the drugs in the small intestine or terminal part of the colon.[9,10] The patho-physiological state of an individual will have a significant impact on the performance of these time-dependent systems. Patients with irritable bowel syndrome and ulcerative colitis exhibited accelerated transit through different regions of the colon.[11,12]

The best alternative approach for colon specific drug delivery is the use of carriers that are degraded exclusively by colonic bacteria. Most of the carrier based systems provide controlled delivery of drugs in matrix and/or reservoir type systems, which pose problems of bioavailability fluctuation due to pH variations.[13,14] Moreover, the release of drugs from matrix and/or reservoir type systems is affected by hydrodynamic conditions of the body.

Osmotic drug delivery system (ODDS) utilizes the principle of osmotic pressure for controlled delivery of drugs.[15] Drug release from these systems is independent of pH and other physiological parameter to a large extent and exhibit significant *in vitro–in vivo* correlation.[16] Drug delivery from ODDS follow zero-order kinetic hence provides better control over *in-vivo* performance. Various types of osmotic pumps have been reported to target the drug to colon for local or systemic therapy.[17—19] These systems were essentially time dependent systems. High variation of gastric retention time makes these systems complicated in predicting the accurate location of drug release.

With all these considerations in mind, we designed microbially activated osmotic delivery systems (MAODS) for colon-targeted delivery of MTZ. Figure 1 shows schematic diagram of MAODS, which consists of an osmotic core (containing drug, osmotic agent and wicking agent), an inner semipermeable membrane (SPM) layer composed of the mixture of cellulose acetate and guar gum as a pore former, and an outer enteric-coating layer. During its transit through the GIT, MAODS remains intact in the stomach due to the enteric-coating layer, but this layer will dissolve in the small intestine, where pH is above 6, and fluid is imbibed into the core due to osmotic pressure gradient across SPM. The con-

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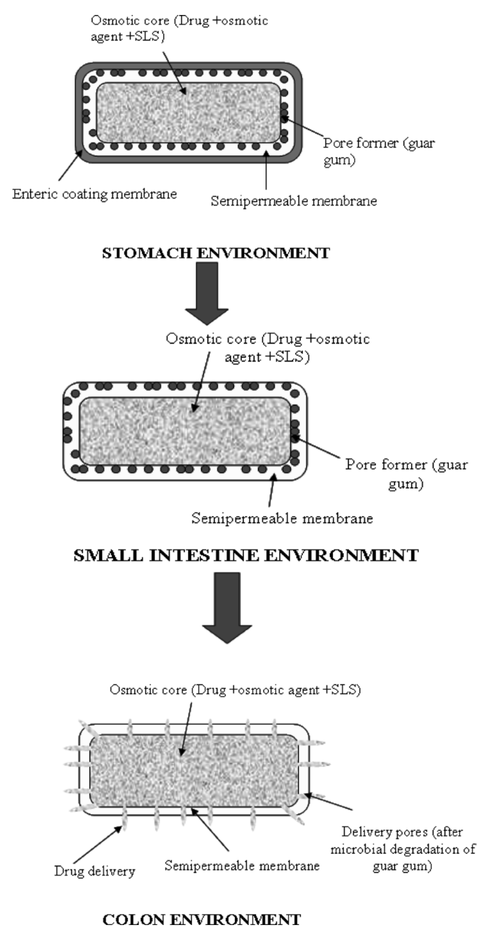

Fig. 1. Schematic Representation of Microbially Activated Osmotic Delivery System (MAODS)

tinuous imbibition of core forms a saturated solution of drug within the device. When MAODS reaches the colon, guar gum (pore former) in the semipermeable membrane is specifically degraded by microflora[20] of the colon and thereby results in an *in situ* formation of a delivery pores. The saturated solution of drug is delivered from these delivery pores at a relatively constant release rate for up to 12 h in the colon.

Experimental

Materials Metronidazole (97.9% purity) was a gift sample from J. B. Chemicals & Pharmaceutical Ltd., Mumbai, India. Eudragit S-100 was obtained as a gift sample from Ranbaxy Laboratories Ltd., Gurgaon, India. Following chemicals and excipients were purchased from commercial sources and used as such: cellulose acetate (39.8% acetylation), polyvinyl pyrrolidone (PVP K-30), microcrystalline cellulose (MCC) (Avicel PH-101), magnesium stearate, talc, sodium chloride (each from CDH, Delhi, India) fructose, mannitol, acetone, methanol, acetonitrile, triethanolamine, methanol, ethanol (all from Qualigens Fine Chemicals, Mumbai, India). PEG-400, sodium lauryl sulphate, guar gum, disodium hydrogen orthophosphate, orthophosphoric acid (all from S.D. Fine Chemicals, Mumbai, India).

Methods Preparation of Core Tablets: Before initiating formulation development, compatibility of MTZ with different excipients was tested using the techniques of DSC (DU-PONT, Model 9900, U.S.A), and FT-IR (SHIMADZU, Model 8400S, Tokyo, Japan). Excipients used in the final formulation were found to be compatible with MTZ. Core tablets of MTZ (150 mg)[21] were prepared by direct compression and batch size was kept as 100 tablets. Formula of different core formulations of MTZ is listed in Table 1. MTZ was mixed with fructose and mannitol for 10 min. After passing this mixture through #30 mesh sieve, sodium lauryl sulphate (SLS) and microcrystalline cellulose (MCC) were added in geometric dilution and mixing continued for additional 10 min. To this mix talc and magnesium stearate each passed through #60 mesh sieve were added and mixing continued for additional 10 min. The blend was then directly compressed into tablets having average weight of 360 mg using a single station tablet punching machine (Manesty E-2, London, U.K.) fitted with 8 mm round standard concave punches. The punched tablets were of 6 ± 0.38 kg/cm^2 hardness on Monsanto hardness tester (Campbell Electronics Mumbai, India). The drug content of tablets was found to be within the limit of 97.98—102.36%.

Table 1. Formula for Different Batches of Core Formulation

Ingredients (mg/tablet)	Batch number						
	I	II	III	IV	V	VI	VII[a]
Metronidazole	150	150	150	150	150	150	150
Mannitol	100	70	70	70	70	58	81
Fructose	—	80	80	80	80	66	93
SLS	—	—	15	25	35	25	25
PVP	15	15	15	15	15	15	15
MCC	91	41	26	16	6	42	—
Talc	2	2	2	2	2	2	2
Magnesium stearate	2	2	2	2	2	2	2

MCC=microcrystalline cellulose, PVP=polyvinylpyrrolidone, SLS=sodium lauryl sulphate. *a*) Batch with tablet weight of 368 mg.

Table 2. Composition of SPM Coating Solutions

Ingredients	Coat code		
	A	B	C
Cellulose acetate (g)	2.00	2.00	2.00
PEG-400 (g)	0.39	0.39	0.39
Guar gum (g)	0.20	0.40	0.60
Acetone (ml)	80	80	80
Methanol (ml)	20	20	20

Preparation of Osmotic Delivery System (ODS): ODS were prepared by coating of core tablets with a SPM in a conventional laboratory coating pan having outer diameter of 20 cm (Scientific Instrument, New Delhi, India) fitted with three baffles placed at angle of 120°.[22] The composition of coating solutions used for coating of MTZ tablets is given in Table 2. Various components of coating solution were added to solvent mixture in sequential manner. The component added first was allowed to dissolve before next component was added. Coating process was started on a batch of 100 tablets; pan speed was maintained at 20 rpm and hot air inlet temp. was kept at 38—42 °C. The manual coating procedure based on intermittent spraying and coating technique was used with spray rate of 4—5 ml/min.[22] Coat weight and thickness were controlled by the volume of coating solution consumed in coating process.[21] Coating was continued until desired coat thickness (90 μm) was obtained on the core tablets. In all the cases coated tablets were dried at 50 °C for 10 h before further evaluation.

Preparation of Microbially Activated Osmotic Delivery Systems (MAODS): MAODS were prepared by enteric coating of ODS with Eudragit S-100 (10% w/v in ethanol) to give enteric coat thickness of 50, 70, and 90 μm coded as ET1, ET2 and ET3 respectively. Coating process was started on a batch of 100 SPM coated tablets at a time and exactly same method was followed as explained above with hot air inlet temp. was kept at 40—42 °C and spray rate of 2—4 ml/min. Coating was continued until desired coat thickness was obtained on the SPM coated core tablets. In all cases coated tablets were dried at 50 °C for 4 h before further evaluation.

Evaluation of Developed Formulations Evaluation of Powder Blend: The bulk and tap density of the powdered blend was determined using USP method II on tap density tester (ETD-1020, Electrolab, India) and compressibility index and Hausner ratio were calculated.

Evaluation of Core and Coated Tablets: The core tablets were evaluated for weight variation, hardness, friability, thickness and diameter. The ODS were also evaluated for weight variation, hardness, thickness and diameter of tablets. The developed MAODS were evaluated for diameter and thickness of tablets.

Thickness and diameter of the core and coated tablets were measured using screw gauze (Ultra Science Aid, Mumbai, India). Hardness of randomly selected tablets was tested using Monsanto hardness tester. Friability of core tablets and MAODS was carried out on a Roche friabilator (Electrolab, Mumbai, India) using 20 accurately weighed tablets.

In-Vitro Drug Release Study: The developed formulations ($n=3$) of MTZ were subjected to *in-vitro* drug release studies. These studies were carried out using a USP XXIV dissolution rate test apparatus (Apparatus 1, 50 rpm, 37 °C) (Campbell Electronics, Mumbai, India). The tablets were tested for drug release for 2 h in simulated gastric fluid (SGF) (pH 1.2, 900 ml) as the average gastric emptying time is about 2 h. Then the dissolution medium was replaced with simulated intestinal fluid (SIF) (phosphate buffer pH 7.4, 900 ml) and tested for drug release for 3 h as the average small intestinal transit time is about 3 h. Then the dissolution medium was further replaced with 100 ml only of simulated colonic fluid (SCF) (pH 6.8 phosphate buffered saline containing 4% w/v of rat caecal contents) contained in 200-ml beaker, and immersed in water maintained in 900 ml vessel, which in turn was in the water bath of the apparatus.[23] As the caecum is naturally anaerobic, the experiment was carried out with continuous CO_2 supply into the beaker. The dissolution study was continued for another 8 h. At various time intervals, 5 ml of the dissolution sample was withdrawn without a pre-filter. The samples were centrifuged, the supernatant filtered through a 0.45-mm membrane filter and the filtrate was analyzed for MTZ by modified validated HPLC method at 317 nm.[24]

Release profiles of various batches were compared using model independent pair wise approach, which include the calculation of 'difference factor' $f1$ and 'similarity factor' $f2$. The two release profiles were considered to be similar if $f1$ value was lower than 15 (between 0 to 15) and $f2$ value was more than 50 (between 50 to 100). Release profiles were also compared using mean dissolution time (MDT), which was calculated using following equation[25]:

$$\mathrm{MDT} = \frac{\sum_{j=1}^{n} \hat{t}_j \Delta M_j}{\sum_{j=1}^{n} \Delta M_j} \tag{1}$$

Where j is the sample number, n is the number of dissolution sample times, t_j is the time at mid point between t_j and $t_{(j-1)}$, and ΔM_j is the additional amount of drug dissolve between t_j and $t_{(j-1)}$. One way analysis of variance test (ANOVA) was performed to check whether there is significant difference among the different formulations.

In this study mean dissolution time for 50% drug release ($MDT_{50\%}$) was used for comparison of release profiles from different batches.

For content uniformity testing, accurately weighed tablets ($n=20$) were dissolved in 500 ml of distilled water.[26] The samples were sonicated for 30 min and filtered through 0.45 μm nylon membrane filter. The filtered samples, after appropriate dilution with mobile phase, were analyzed at 317 nm using HPLC[24] (CECIL HPLC system, Mumbai, India).

In addition, the developed formulations were subjected to various tests as follows.

Effect of pH: To study the effect of pH and to assure a reliable *in-vivo* performance of the developed formulations, release studies of the optimized formulations were also conducted according to pH change method after 5 h of release studies already conducted in SGF and SIF. The release media in pH change method were pH 7.4 for first 2 h, pH 6.8 for next 2 h, pH 4.5 for next 2 h followed by pH 1.2 for last 2 h with 4% rat caecal content in each case. The samples (5 ml) were withdrawn at predetermined intervals and analyzed after filtration through 0.45-μm nylon membrane filters.

Effect of Agitation Intensity: To study the effect of agitation intensity of the release media, release studies of the optimized formulation were carried out in dissolution apparatus at various rotational speeds. Dissolution apparatus used was USP-XXIV type I (rotating basket) at 50, 100, and 150 rev./min. In another experiment, stirred and stagnant conditions were induced in a single run using USP-XXIV apparatus I. The rotational speed was kept at 50 rev./min (stirred conditions), which, however, was stopped intermittently to induce the stagnant conditions. The protocol used was intermittent stirred and stagnant condition for period of 2 h up to 12 h. Samples were withdrawn at predetermined intervals and analyzed after filtration through 0.45-μm nylon membrane filters.

Effect of Osmotic Pressure of Release Medium: In order to confirm the mechanism of drug release, release studies of the optimized formulation were conducted in media of different osmotic pressure. To increase the osmotic pressure of the release media, sodium chloride (osmotically effective solute) was added in SIF and the pH was adjusted to 6.8±0.05. Release studies were carried out in 900 ml of media using USP-XXIV dissolution apparatus I (50 rev./min). To avoid any interference in the analysis by sodium chloride, residual drug analysis methodology was utilized for construction of release profile. At predetermined time points, tablets were withdrawn from vessel, cut open, and the contents dissolved in 250—500 ml of SCF. The samples were analyzed to determine the residual amount remaining in the tablet. Accuracy of this method was checked in SCF, where results after direct measurement of MTZ into the release media were similar to the results of residual drug analysis method.

HPLC Analysis: *In-vitro* analysis of drug samples was done on CECIL HPLC system equipped with adept series dual piston pump CE-4100, manual injector CAPLUGS RC-11 and adept series variable wavelength UV/VIS detector CE-4201. Reverse phase HPLC method was carried out using phenomenex C-18 column (4.6×250 mm, 5 μm particle size) at 25 °C. The optimized mobile phase composition was Di-sodium hydrogen orthophosphate (0.05 M)–acetonitrile–triethanolamine (89.9 : 10 : 0.1) and pH was adjusted to 7.0) at flow rate of 1.5 ml/min. Injected volume was 20 μl and detection was performed at 317 nm using a UV/VIS detector.[24]

Burst Strength: Burst strength of the exhausted shells, after 13 h of dissolution, was determined to assure that the tablets would maintain their integrity in the GIT. Burst strength was determined as the force required to break/rupture the shells after dissolution studies. The texture analyzer (Stable Micro systems, TAX T2i, England) with a 5 kg load cell and 25 mm aluminum cylindrical probe was utilized for this purpose. Test speed of 0.8 mm/s was selected and the distance moved was set at 2 mm.

Scanning Electron Microscopy Studies (SEM): In order to elucidate the mechanism of drug release from developed formulations, surface of coated tablets, both before and after dissolution studies, was studied using scanning electron microscope (SEM). The samples were placed on a spherical brass stub (12 mm diameter) with a double backed adhesive tape. The tablets (coated tablets before dissolution studies) were mounted as such on the specimen stub. On the other hand, small sample of the coating membrane was carefully cut from the exhausted shells (after 13 h of dissolution studies) and dried at 50 °C for 6 h. The mounted samples were sputter coated for 5 to 10 min with gold using fine coat ion sputter (JEOL, JFC-1100, Japan) and examined under SEM (JEOL, JSM-6100, Japan).

Reproducibility Study The reproducibility of the manufacturing procedure was confirmed by preparing three repeat batches of optimized formulation on three different occasions. Drug release characteristics of batches were conducted under similar conditions and were compared with previous release profiles of the same batches.

Accelerated Stability Studies Optimized formulations of TRH were packed in strips of 0.04 mm thick aluminum foil laminated with PVC. The packed formulations were stored in ICH certified stability chambers (NSW-175, Narang Scientific Work, New Delhi, India) maintained at 40 °C and 75% RH for 3 months. The samples were withdrawn periodically and evaluated for drug content, hardness, burst strength, and release studies.

Results and Discussion

The most promising of the colonic drug delivery systems are those that depend on enzymatic action of colonic bacteria on polysaccharides. The polysaccharides that are under investigation as carriers for colon targeted drug delivery include pectin, amylose and chitosan. Recently guar gum has been reported as a potential carrier for colon targeted delivery.[27] Based on this information MAODS of MTZ were developed for effective and safe therapy for intestinal amoebiasis. *In-vitro* release studies showed that guar gum as a pore former in the semipermeable membrane of MAODS is specifically degraded by microflora of the colon and thereby results in an *in situ* formation of a delivery pores. The saturated solution of drug is delivered from these delivery pores at a relatively constant release rate for up to 12 h in the colon.

Effect of Wicking Agent (Sodium Lauryl Sulphate) In initial trial core tablet of MTZ (batch-I) was coated with coating composition B and enteric coated with ET1 (formulation code batch-IC/ET1). Result of release studies showed that only 18% of drug was delivered in 13 h. This phenome-

non could be expected either because of low osmotic pressure of core formulation or due to low solubility of MTZ. To increase the osmotic pressure of core compartment, fructose (335 atm. of saturated solution)[16] was added (formulation code batch-IIC/ET1). This approach was also unsuccessful, as there was only 28% of drug delivered after 13 h of release study.

Osmotic pumps *per se* are suitable for delivery of drugs having intermediate water solubility.[28,29] It has been reported that in case of highly water soluble drugs, meaningful release rates may not be obtained using elementary osmotic pump (EOP) or controlled-porosity osmotic pump (CPOP).[30] This is because the kinetics of osmotic drug release is directly related to solubility of drug within the core. Assuming a tablet core of pure drug, the fraction of drug released with zero-order kinetics is given by;

$$F(z) = 1 - \frac{S}{\rho} \quad (2)$$

where $F(z)$ is the fraction released by zero-order kinetics, S the drug's solubility (g/cm^3), and ρ the density (g/cm^3) of the core tablet. Drugs with a solubility of ≤ 0.05 g/cm^3 would be released with $\geq 95\%$ zero-order kinetics according to Eq. 2. However, the zero-order release rate would be slow according to Eq. 3, due to the small osmotic pressure gradient.

$$\frac{dm}{dt} = \frac{A}{h} \sigma L_{\mathrm{p}} (\Delta\pi - p) C \quad (3)$$

Equation 3 describes drug release from osmotic pumps, where dm/dt is the drug delivery rate; A and h the membrane area and thickness, respectively; C is the concentration (or the solubility, when excess of drug is present in the core) of drug in the dispensed fluid, $\Delta\pi$ is the osmotic pressure difference across the film, σL_{p} is the hydraulic permeability of the membrane and p is the hydrostatic pressure within the core compartment.[31]

According to Eq. 2, highly water-soluble drugs would demonstrate a high release rate that would be zero-order for a small percentage of the initial drug load. Thus, the intrinsic water solubility of many drugs might preclude them from incorporation into an osmotic pump. However, it is possible to modulate the solubility of drugs within the core, and thus extend this technology for delivery of drugs, which otherwise may be poor candidates for osmotic delivery.

The solubility of MTZ in water is reported to be 10 mg/ml,[32] which is less than desired solubility requirement (50—300 mg/ml) for osmotic delivery system.[30] In order to get the desired release from the developed formulation SLS was added in core formulation to modulate the solubility of MTZ within the core. Inclusion of SLS (non-swelling wicking agent) in core is expected to draw more water in the porous network of delivery device. Hence there will be more solubilization of drug within the core.[26] Three batches were prepared in which concentration of SLS was varied. Batch-III, IV, V coated with SPM coating composition C and enteric coat ET1 coded as batch-IIIC/ET1, IVC/ET1, and VC/ET1 containing 4.16%, 6.94%, and 9.72% w/w of SLS respectively were prepared. *In-vitro* release profiles of all three batches were compared with batch-IIC/ET1 (without SLS) and in Fig. 2. It is clearly evident that with the increase in concentration of SLS there was significant increase

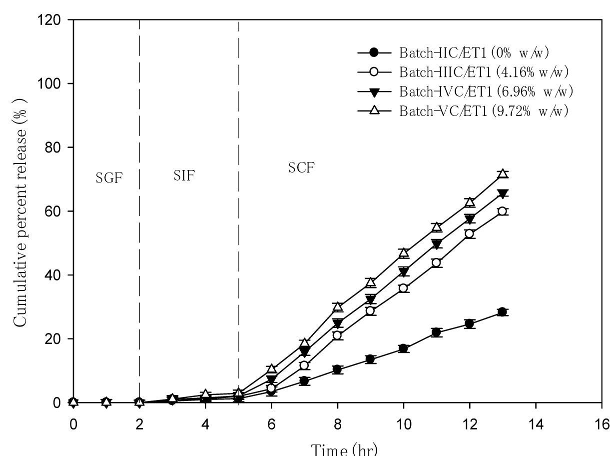

Fig. 2. Profiles Showing the Effect of Wicking Agent (SLS) on MTZ Release from Developed Formulations

Bars represent ± S.D. ($n=3$).

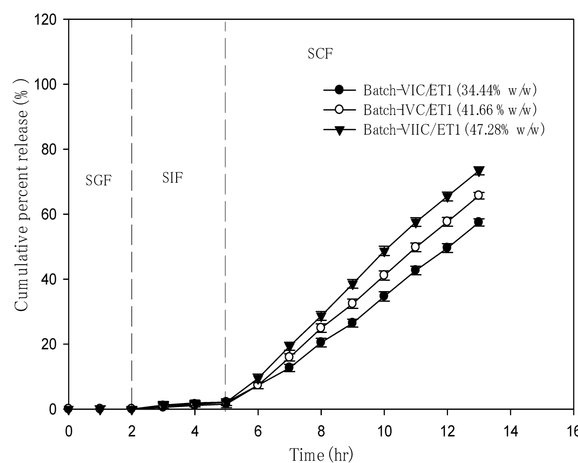

Fig. 3. Profiles Showing the Effect of Level of Osmotic Agents on MTZ Release from Developed Formulations

Bars represent ± S.D. ($n=3$).

($p<0.05$) in rate and extent of drug release from developed formulations. The $\mathrm{MDT}_{50\%}$ between different formulations (not achieved, 8.634, 7.758, and 6.662 h for batch-IIC/ET1, IIIC/ET1, IVC/ET1, VC/ET1, respectively) was found to be statistically significant ($p<0.05$).

Effect of Level of Osmotic Agent To study the effect of level of osmotic agents on MTZ release, core tablets of MTZ were prepared with different levels of osmotic agents (fructose : mannitol in ratio of 1 : 0.8). Batch-VI, VII having 34.44%, and 47.28% w/w of osmotic agent respectively were prepared and coated with SPM coating composition C and enteric coated with ET1 coded as batch-VIC/ET1, VIIC/ET1. *In-vitro* release profiles of these formulations were compared with batch-IVC/ET1 (containing 41.66% w/w osmotic agent) in Fig. 3. It is clearly evident that with increase in level of osmotic agent there is significant ($p<0.05$) increase in drug release. The $\mathrm{MDT}_{50\%}$ was found to be 6.659, 7.758, and 8.724 h for formulation containing 34.44%, 41.66% and 47.28% w/w of osmotic agent respectively. There was statistically significant difference ($p<0.05$) between the different formulations. Batch VIIC/ET1 had shown maximum MTZ release followed by IVC/ET1 and VC/ET1 which gave lowest

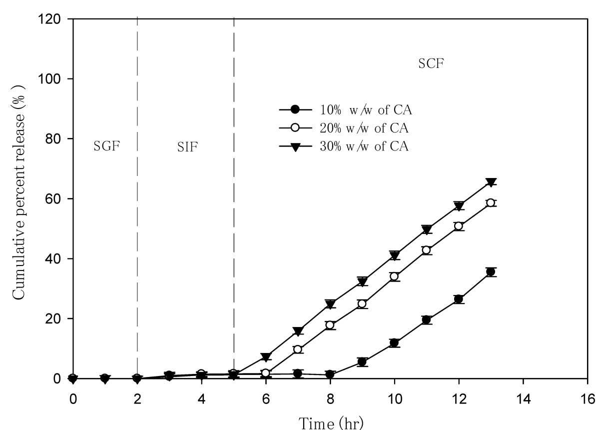

Fig. 4. Profiles Showing the Effect of Level of Pore Former (Guar Gum) on MTZ Release from Developed Formulations

Bars represent±S.D. ($n=3$).

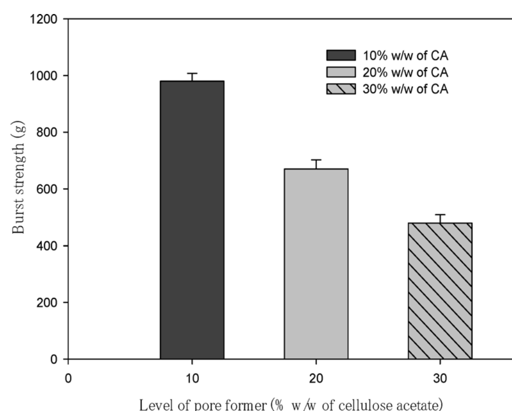

Fig. 5. Bar Diagram Showing the Dependance of Burst Strength of Membrane (90 mm of SPM) on Level of Pore Former (Guar Gum) in Batch-IVC/ET1)

Bars represent±S.D. ($n=3$).

release. This in accordance with earlier report which states that level of osmotic agent has direct effect on rate and extent of drug release.[29)]

Effect of Pore Former (Guar Gum) To study the effect of level of pore former (guar gum), core formulation (batch-IV) of MTZ were coated with SPM coating formulation A and B containing 10% and 20% w/w (of cellulose acetate) level of guar gum respectively and enteric coated with ET1 coded as batch-IVA/ET1 and IVB/ET1. Release profiles of these formulations in comparison with batch-IVC/ET1 (containing 30% w/w of guar gum) are shown in Fig. 4. It is clearly evident that level of pore former has direct effect on the drug release. As the level of guar gum increases the membrane becomes more porous due to degradation of larger amount of guar gum by microflora of SCF resulting in higher drug release. $MDT_{50\%}$ between different formulations (8.587, 7.754 and 6.158 h for formulation with 10%, 20% and 30% w/w of guar gum, respectively) was found to be statistically significant ($p<0.05$). Burst strength of the exhausted shells was also affected by the level of pore former. With the increase in level of guar gum, the membrane became more porous after exposure to SCF, leading to a decrease in its strength. Effect of level of guar gum on burst strength is shown in Fig. 5.

Another parameter affected by the concentration of pore forming agent was the lag time of drug release. Before SCF dissolution study, it was expected that there was a lag time of 5 h to reach specific colon region, which meant that the release of drug was only activated by colonic bacteria of SCF. However, in the SCF dissolution study, there should not be any lag time. The concentration of guar gum in the membrane might be the key factor to this lag time. The lag time was inversely related to the initial level of pore former (guar gum,) in the membrane. The lower concentration of pore forming agent (10% w/w of CA, SPM coating A) showed longer average lag time (8±0.5 h) and the higher concentration of pore forming agent (30% w/w of CA, SPM coating C) showed shorter average lag time (5±0.4 h) in complete dissolution study. Whereas SPM coating B (20%w/w of CA of pore former) showed lag phase of 6±0.4 h.

Effect of Thickness of SPM To study the effect of coat thickness of SPM on drug release core tablet of MTZ (batch-IV) were coated with coating composition C so as to give different coat thickness (70, 90, 110 μm) and enteric coated with coat ET1. Release profiles of MTZ from these formulations are shown in Fig. 6. Profiles indicated that drug release was decreased with the increase in coat thickness of SPM. The increase of SPM thickness resulted in an increased resistance of SPM to water imbibition, causing a rate of decreased water imbibition consequently causing a decrease in rate of liquefaction/dissolution of drug in core, and ultimately resulted in decline in MTZ release. $MDT_{50\%}$ between different formulations (8.762, 7.758, 6.212 for formulation with SPM coat thickness of 70, 90, 110 μm, respectively) was found to be statistically significant ($p<0.05$). No bursting of the systems was observed during the dissolution run in any of the formulations. In addition, exhausted tablets (after 13 h of dissolution studies) were evaluated for burst strength to assure that the tablets maintain their integrity in GIT and do not lead to dose dumping. Figure 7 shows the dependence of burst strength of the exhausted shells on coat thickness. The strength of mechanical destructive force in the GIT of human and dog has been reported to be 1.9 N (approximately 190 g) and 3.2 N (approximately 320 g), respectively.[33,34)] It has been reported that osmotic pumps having the burst strength in the range of 500—600 g were intact in the GIT of

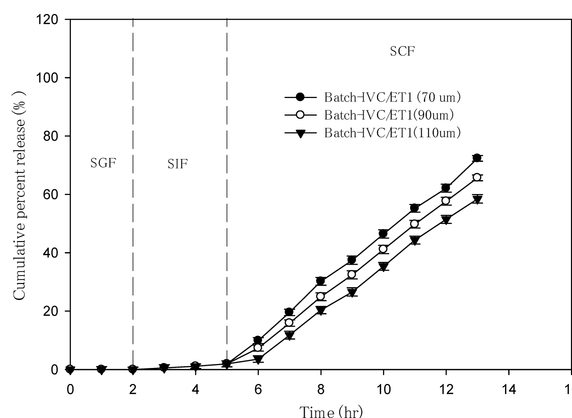

Fig. 6. Profiles Showing the Effect of SPM Coat Thickness on MTZ Release from Developed (Batch-IVC/ET1) Formulations

Bars represent±S.D. ($n=3$).

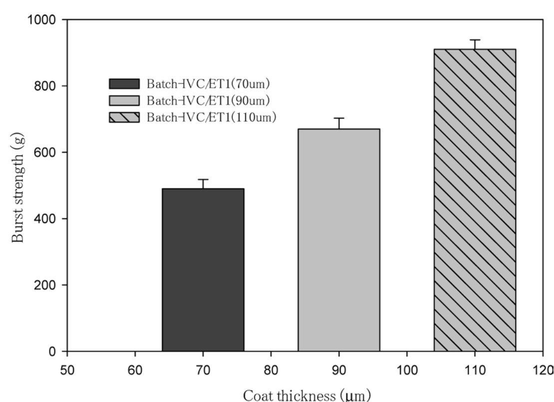

Fig. 7. Bar Diagram Showing the Dependance of Burst Strength on Coat Thickness of Membrane

Bars represent±S.D. ($n=3$).

dogs while those having burst strength of around 200 g were compromised. In all developed formulations, the value is much higher than the mechanical destructive forces in GIT, thus assuring that the formulations can be expected to remain intact in GIT without any incidence of dose dumping.

Effect of Thickness of Enteric Coat In all the above studies thickness of enteric coat was kept constant at 50 μm. To study the effect of thickness of enteric coat on MTZ release from MAODS. Formulations of batch-IV coated with SPM coating composition C were enteric coated to give enteric coat of thickness 50 μm, 70 μm, and 90 μm coded as batch-IVC/ET1, batch-IVC/ET2 and batch-IVC/ET3 respectively. Release profiles of MTZ from these formulations are showed insignificant ($p>0.05$) effect of enteric coat thickness on rate and extent of drug release. This effect may be explained as enteric polymer (Eudragit S-100) dissolved too quickly in SIF but guar gum present in SPM was not dissolved, delivery pores were not formed and therefore it could not bring any change in drug release of MTZ.

Release Kinetics and Selection of Optimized Formulation In order to investigate the drug release kinetics, release data of promising batches were fitted to models representing zero-order, first-order and Higuchi's square root of time. The data were analyzed by the regression coefficient method and regression coefficient (R^2) value of all batches is given in Table 3. On the analysis of regression coefficient value of all batches, it was found that batch-IVC/ET1 best followed the zero-order kinetic ($R^2=0.9997$), confirming that the release from this formulation is much closer to desired release, hence selected as optimized formulation.

Performance Evaluation of the Optimized Formulation The optimized formulation was evaluated for various pharmacopoeial and non-pharmacopoeial tests, results of which are listed in Table 4. The powder blend was free flowing as demonstrated by the values of compressibility index (less than 15) and Hausner ratio (less than 1.25). Other parameters for the uncoated and coated tablets were also within limits. Exhausted shells, after dissolution, were visually observed for any imperfection or cracks in the coating. There were no visible cracks in the coating and it was found to be intact in all the batches after 13 h of dissolution studies. The burst strength of the exhausted shell was found to be much more than the reported mechanical destructive forces in the GIT of

Table 3. Release Kinetics of Metronidazole from Developed Formulations

Batch number	Regression coefficient (R^2)		
	Zero-order	First order	Higuchi
Batch-IIC/ET1	0.9942	0.9888	0.9650
Batch-IIIC/ET1	0.9982	0.9904	0.9857
Batch-IVC/ET1	0.9997	0.9835	0.9914
Batch-VC/ET1	0.9983	0.9805	0.9868
Batch-VIC/ET1	0.9980	0.9799	0.9689
Batch-VIIC/ET1	0.9981	0.9836	0.9885

Table 4. Properties of the Powdered Blend, Core Tablets, and Final Coated Tablets of the Optimized Formulation (Batch-IVC/ET1)

Parameters	Value±S.D.
Bulk density[a)] (mg/cm^3)	452
Tap density[a)] (mg/cm^3)	531
Compressibility index[a)] (%)	14.69
Hausner ratio[a)]	1.174
Tablet weight (mg, $n=10$)	
Core tablet	360±5.21
SPM coated tablet	376±4.62
Enteric coated tablet	384±4.24
Thickness (mm, $n=10$)	
Core tablet	4.32±0.02
SPM coated tablet	4.44±0.02
Enteric coated tablet	4.52±0.06
Diameter (mm, $n=10$)	
Core tablet	8.11±0.11
SPM coated tablet	8.22±0.02
Enteric coated tablet	8.26±0.04
Hardness (kg/cm^2)	
Core tablet	6.12±1.12
SPM coated tablet	10.24±1.48
Enteric coated tablet	11.28±1.58
Friability[b)] (%)	0.096
Content uniformity[c)] (%, $n=20$)	102.46±2.24

a) Properties of powdered blend; *b*) property of the core tablet; *c*) property of final coated tablet.

humans, assuring that the formulation would be intact in GIT.[33,34)]

In order to assure reliable *in-vivo* performance and to study the effect of pH on drug release, the release studies of optimized formulation (batch-IVC/ET1) were conducted according to pH change method and compared with release profile of MTZ in SCF with 4% rat caecal content after 5 h of release studies in SGF and SIF. Figure 8 shows that release profile of MTZ from batch-IVC/ET1 formulation is similar in both the media. The $f1$ and $f2$ values of batch-IVC/ET1 were found to be 2.65 and 91.85, respectively, taking the release profile in SCF as the reference.

Drug release from osmotic pumps, to a large extent, is independent of agitation intensity of the release media. Two experiments were conducted to study the effect of this parameter. In the first experiment, release studies of batch-IVC/ET1 formulation were carried out in USP-XXIV dissolution apparatus type I at varying rotational speed (50, 100, 150 rev./min). Figure 9 shows that the release profile of MTZ from the developed formulations is fairly independent of the agitation intensity of the release media and hence, it can be expected that the release from the developed formulations will be independent of the hydrodynamic conditions of the

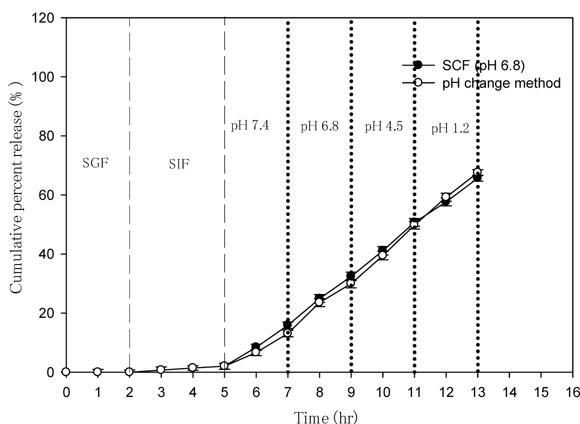

Fig. 8. Profiles Showing the Effect of pH on MTZ Release from Developed Formulations (Batch-IVC/ET1)

Bars represent ± S.D. ($n=3$).

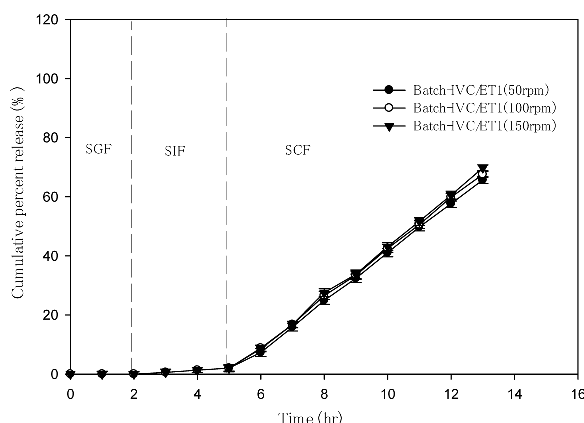

Fig. 9. Profiles Showing the Effect of Agitation Intensity on MTZ Release from Developed Formulations

Bars represent ± S.D. ($n=3$).

absorption site. The $f1$ and $f2$ values of batch-IVC/ET1were found to be 4.22 and 85.09 (between 50 and 100 rev./min), 3.71 and 85.78 (between 100 and 150 rev./min), and 7.77 and 73.33 (between 50 and 150 rev./min), respectively. In the second experiment, stirred and stagnant conditions were induced in the same run. Release studies of batch-IVC/ET1 formulations were carried out in USP-XXIV apparatus (at 50 rev./min). The stirring, however, was stopped after fixed time intervals so as to induce stagnant conditions. Release rates were calculated and compared with those obtained at 50 rev./min (stirred conditions). It was observed that the release rate is similar in both the experiments. Finally, it was concluded that drug release from the developed osmotic pumps is independent of the agitation intensity of the release media.

To study the effect of osmotic pressure, release studies of the optimized formulation (batch-IVC/ET1) were conducted in media of different osmotic pressure (19.45, 47.37, 100.24 atm.) after initial 5 h of dissolution study. The results of release studies in media of different osmotic pressure showed that the drug release is highly dependent on the osmotic pressure of the release media. MTZ release from the formulations decreased as the osmotic pressure of the media increased (Fig. 10). Hence it was concluded that osmotic pumping is the major mechanism governing drug release from developed formulations.

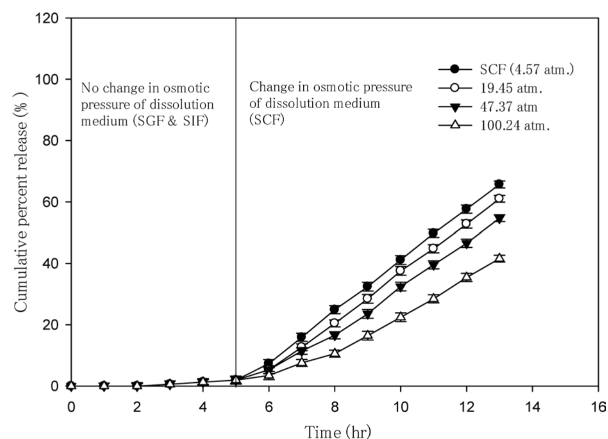

Fig. 10. Profiles Showing the Effect of Osmotic Pressure of Release Media on MTZ Release from Optimized Formulations (Batch-IVC/ET1)

Bars represent ± S.D. ($n=3$).

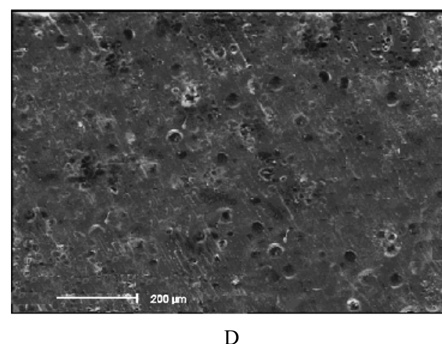

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Fig. 11. SEM Micrograph Showing Membrane Structure of Formulation Batch-IVA/ET1 after SCF Dissolution

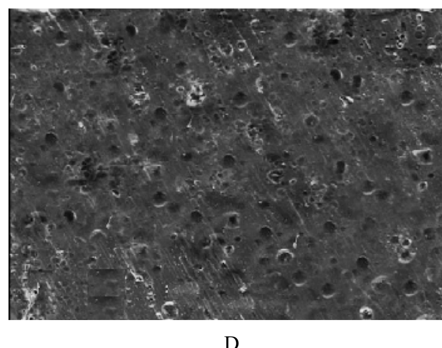

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Fig. 12. SEM Micrograph Showing Membrane Structure of Formulation Batch-IVB/ET1 after SCF Dissolution

Mechanism of Drug Release To investigate the changes in the membrane structure, and mechanism of drug release surface of coated tablets (both before and after dissolution studies) was studied using SEM. Figures 11—13 show SEM micrographs of membrane surface of formulations (batch-IVA/ET1, IVB/ET1, and IVC/ET1 containing 10%, 20% and 30%w/w of guar gum, respectively) with SPM thickness of 90 μm and enteric coat thickness of 50 μm both before and after dissolution studies. Figures 11, 12, 13A—D represent the SEM micrograph of membrane before dissolution study (A), after dissolution studies in SGF (B), SIF (C) and after SCF (D) dissolution studies for batch-IVA/ET1, IVB/ET1 and IVC/ET1, respectively.

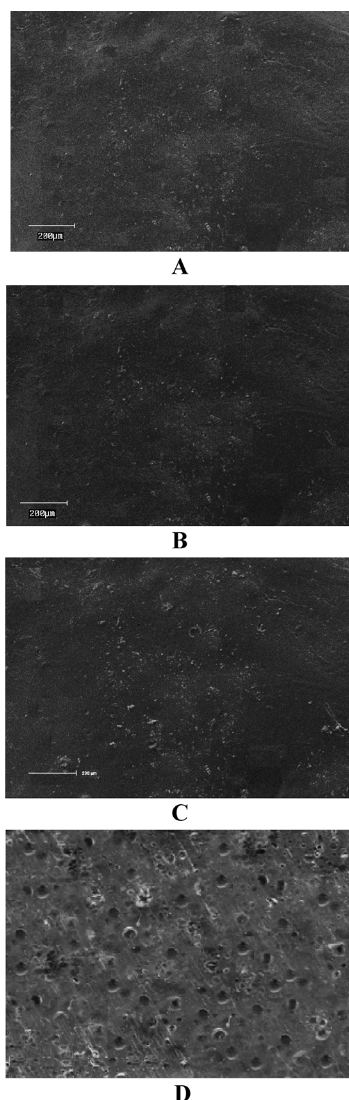

Fig. 13. SEM Micrograph Showing Membrane Structure of Formulation Batch-IVC/ET1 (A) before dissolution study, (B) after SGF dissolution, (C) after SIF dissolution, (D) after SCF dissolution

Before dissolution studies, no porous membrane structure was observed with different levels of pore former (guar gum) (Fig. 13A). Figure 13B showed SEM micrograph of membrane after SGF dissolution studies, the micrograph did not show any evidence of pore formation in the membrane. After dissolution study in SIF, the enteric membrane was dissolved, however SEM micrograph did not show (Fig. 13C) any evidence of pore formation. This might be due to insolubility or nondegradation of guar gum in intestinal fluid. After exposure to SCF release medium, micrograph showed presence of *in-situ* pores in the membrane (Figs. 11D, 12D, 13D) which acted as an exit pores for drug release.

When comparison was made between the membrane containing 10%, 20% and 30% w/w of guar gum (IVA/ET1, IVB/ET1, and IVC/ET1, respectively), it was found that later (Fig. 13D) become more porous after SCF dissolution study.

Reproducibility Study The reproducibility of the manufacturing procedure was confirmed by preparing three repeat batches of the final optimized formulation (batch-IVC/ET1) on three different occasions. Release studies were conducted according to procedure described earlier and similar release

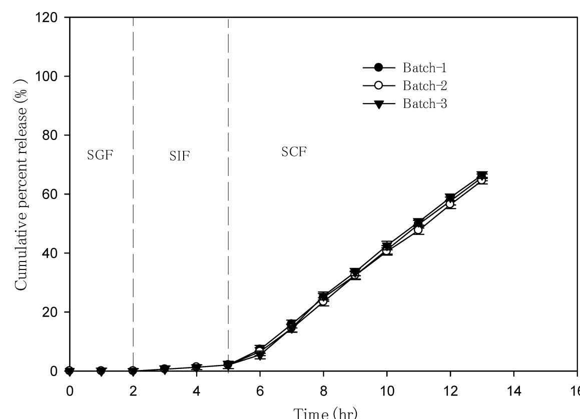

Fig. 14. Profiles Showing the Reproducibility of Manufacturing Procedure on MTZ Release from Three Repeat Batches of Optimized Formulation (Batch-IVC/ET1)

Bars represent ± S.D. ($n=3$).

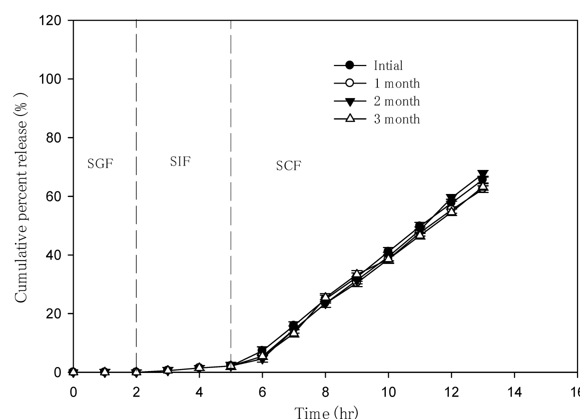

Fig. 15. Profiles Showing the Stability of Optimized Formulation (Batch-IVC/ET1) during 3 Months of Storage at 40 °C and 75% RH

Bars represent ± S.D. ($n=3$).

profiles were obtained from all batches (Fig. 14) demonstrating that the manufacturing procedure is reproducible. The $f1$ and $f2$ values were found to be 4.11 and 85.07 (between batches 1 and 2), 3.26 and 89.46 (between batches 1 and 3), and 5.23 and 81.68 (between batches 2 and 3), respectively.

Accelerated Stability Study The stability studies data indicated that the formulations were found stable as non-significant ($p>0.05$) difference were observed in terms of drug content and release studies prior and after storage (Fig. 15, Table 5). In all the cases, the burst strength was higher than the reported values of mechanical destructive forces in the GIT, ensuring that the formulations to be intact in GIT without any incidence of dose dumping.

Conclusions

A microbially activated osmotic pump (MAODS) for colonic delivery of metronidazole was developed. The effect of various formulation variables was studied to optimize the release profile. Drug release was inversely proportional to SPM thickness, but directly related to the level of pore former, osmotic agent and wicking agent. The release from the developed formulations was independent of pH, and agitation intensity. MTZ release from the developed formulation was inversely related to osmotic pressure of release medium

Table 5. Evaluation of Batch-IVC/ET1 Formulation during 3 Months of Storage at 40 °C and 75% RH

Parameter	Initial	1 month	2 month	3 month
Drug content (%)	102.60±1.44	99.68±1.87	98.14±1.67	104.60±1.61
Hardness (kg/cm^2)	11.33±1.98	12.98±1.68	12.33±1.48	12.13±1.52
Burst strength (kg)	670.88±1.62	671.675±1.82	675.453±1.42	674.687±1.92
$f1$	—	2.32	4.52	5.12
$f2$	—	92.62	88.62	85.24
$MDT_{50\%}$	7.758	7.774	7.734	7.698

confirming osmotic pumping as the major mechanism of drug release. The thickness of enteric coating could prevent formation of delivery pores before contact with SCF, but had no effect on the drug release. Results of SEM studies showed the formation of pores in the membrane after coming in contact with simulated colonic fluid with the number of pores dependent on initial level of pore former in the membrane. The manufacturing procedure was standardized and found to be reproducible. The developed formulations were found to be stable during 3 months of storage at accelerated stability conditions.

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