Research Article

An *In Vitro* and *In Vivo* Assessment of Okra Gum Matrices for Colon Delivery of Theophylline

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ABSTRACT

Colon targeting would be valuable when such a delay in absorption is therapeutically desirable in treatment of chronic medical conditions like nocturnal asthma. Objective: The major objective was to modulate drug release of prepared matrices to target the nocturnal peak symptoms of asthma. Materials and methods: Colon-specific drug delivery based on a polysaccharide, okra gum, was evaluated using in vitro and in vivo methods. Release kinetics was evaluated by using United States Pharmacopoeia (USP) type I dissolution apparatus. Results and discussion: Dissolution study revealed that okra gum preparation was able to protect the drug from being released under conditions mimicking mouth to colon transit with 27.7 ± 3.1% drug releases. Studies in pH 6.8 phosphate buffered saline (PBS) containing 4% w/v rat cecal contents have demonstrated the susceptibility of okra gum to colonic bacterial enzyme action with consequent drug release. In vivo ingestion in rabbits showed controlled release pharmacokinetic profile of theophylline from the formulation prepared using 400 mg okra gum with C_{max} of 22.2 mcg/mL at 4.5 hours (T_{max}). Conclusion: Thus the study clearly established that okra gum, in the form of matrix former, is a potential carrier for drug targeting to colon.

Keywords: C_{max}, Matrix tablet, Rat cecal contents, Release kinetics, T_{max}

INTRODUCTION

Asthma is a chronic obstructive lung disease reported to be circadian rhythm dependent and characterized by airways inflammation and hyper-reactivity. In most patients, the condition worsens at night with acute exacerbation being most common. Clinical and epidemiological studies verify that asthma is several hundred folds more likely at night than during the day with disturbance of sleep at least once weekly in approximately 75% of those afflicted[1,2]. The possibility of deferring the drug release for a programmed time interval after oral administration of the dosage form is considered as a promising tool for handling, among recently highlighted chronopathologies, especially those presenting nocturnal and early morning symptoms[3].

Colon specific drug delivery would be valuable when such a delay in absorption is therapeutically desirable in treatment of chronic medical conditions like nocturnal asthma. Colon specific drug delivery after oral administration at bed time would be logistically utilized for such diseases because according to which a drug is going to be released from the dosage form when it reaches colon and generally it takes 4-5hours that might be coincide with the acute exacerbation of asthmatic attack in the early morning to produce maximum health benefit and minimum harm.

Many approaches have been attempted for colon targeting[4] and it appears that microbially-controlled systems based on natural polysaccharides[5-7] have the greatest potential for colonic delivery. Polysaccharide based drug delivery systems attracting a lot of attention for drug targeting the colon particularly in terms of site-specificity and safety[8] since these polymers of monosaccharides are found in abundance, have wide availability are inexpensive and are available in a verity of a structures with varied

properties. They can be easily modified chemically, biochemically, and are highly stable, safe, nontoxic, hydrophilic and gel forming and in addition, are biodegradable. These include naturally occurring polysaccharides obtained from plant (guar gum, inulin), animal (chitosan, chondrotin sulphate), algal (alginates) or microbial (dextran) origin. The polysaccrides can be broken down by the colonic microflora to simple saccharides.[9] Therefore, they fall into the category of "generally regarded as safe" (GRAS).

Okra gum is a natural polysaccharide obtained from pods of *Abelmuschus esculentus* which had been used as mini matrix for furosemide and diclofenac sodium tablets[10], sulphafuanidine granules and tablets[11] and investigated as well in release of indomethacin from bioadhesive tablets with carbopol[12]. Besides, this gum had been evaluated as a controlled release agent in modified release matrices, in comparison with sodium carboxymethyl cellulose (NaCMC) and hydroxypropylmethylcellulose (HPMC), using Paracetamol as a model drug[13]. The okra polysaccharide contains the major polysaccharide component differing widely in the molar ratios of galactose, galacturonic acid, and rhamnose[14] and with some fractions of glucose, mannose, arabinose and xylose[15].

In recent years researchers pay much attention to okra polysaccharide in pharmaceutical formulation. In recognition of this, it was thought to design a microbially triggered matrix tablet of okra gum by using theophylline to specifically target the nocturnal peak symptoms of asthma. Theophylline, a bronchodilator that relaxes and opens the air passages to the lungs, making it easier to breathe, was selected as a drug candidate considering its short half life (7-9 hours), good oral bioavailability (96%)[16] and good colonic absorption[17,18]. The system when administered in the evening was programmed to release their drug content after 4-5 h to achieve an elevated theophylline level overnight when the risk of asthma is found to be maximum. The major objective was to modulate drug release of these matrices to target the nocturnal peak symptoms of asthma.

MATERIALS

Okra gum was isolated from okra pods which were purchased from local market. Theophylline was obtained as gift sample from Lifeline Industries Limited, Mumbai. High performance liquid chromatography grade distilled water, acetonitrile and methanol were procured from E. Merck (Mumbai). All other chemicals and reagents used in the study were of analytical grade.

METHODS

Isolation of okra gum

A 3kg weight of fresh Okra pods, from which the stalk and apex of the pods had been removed, was weighed. As the seeds do not contain any mucilage and hence were removed. The okra was sliced with a hand knife, homogenized with two times its weight of 70% (vol/vol) aqueous ethanol at room temperature. The resultant paste was than filtered to get insoluble residue. The residue was washed with double volumes of chloroform/methanol (1/1, vol/vol) with gentle stirring for 30 min to remove low molecular weight (colored) compounds followed by filtration using muslin cloth to get pure residue. The residue was finally washed with acetone and then oven dried for 24h and dried okra powder was preserved for further study.

Compatibility study

DSC spectra of pure drug and mixture of formulation were recorded by dispersion of drug and mixture of formulation in suitable solvent using Differential scanning calorimeter to study drug-excipients compatibility at SICART, Gujarat.

Preparation of matrix tablets

Based on Preliminary study matrix tablets (average weight of 625 mg) of okra gum containing theophylline (200 mg) were prepared by wet granulation method using Poly Vinyl Pyrrolidine K30 (PVPK30) in sufficient quantity of isopropyl alcohol

(IPA) as the binder. Micro crystalline cellulose (MCC) was used as diluent. To check the effect of polymer concentration on drug release pattern, tablets were prepared using different drug to polymer ratios (1:1, 1:1.5, 1:2). The composition of different formulations used in the study is given in Table 1. In all the formulations, polymers were sieved (60#) separately and mixed with theophylline (100#) and MCC (60#). The powders were blended and granulated with PVP K30 in sufficient IPA. The wet mass was past through a mesh (100#) and the granules were dried at 40^0C for 15 min. The dried granules past through a mesh (100#) and were evaluated for their granulation properties. The resultant granules were lubricated with magnesium stearate followed by compression by using 12 mm round, flat, and plain punches on a 10 station tableting machine (Karnavati engineering, Kalol, India).

Characterization of granules[19,20]

Prior to compression granules were evaluated for their micromeritic properties like angle of repose, bulk density, tapped density, Carr's index and Hausner's ratio. Angle of respose was determined by funnel method. Bulk density and tapped density were determined by cylinder method.

The Carr's index (CI) and Hausner's ratio (HR) were calculated using the following equations.

CI = [(Tapped density-Bulk density) / Tapped density] × 100

HR = Tapped density/Bulk density

The drug content in granules was determined by extracting an accurately weighed amount of powdered granules (100 mg) with water. The solution was filtered through 0.45 µm membrane and absorbance was measured at 271 nm after suitable dilution.

Characterization of matrix tablets

The properties of the compressed matrix tablet, such as hardness, friability, weight variation, and content uniformity were determined using reported procedure[21-23]. Briefly, hardness was determined by using Monsanto hardness tester. Friability was determined using Roche friability testing apparatus.

Weight variation

For weight variation test, 20 tablets of each formulation were selected at random and weighed individually. The individual weights were compared with average weight for determination of weight variation.

Content uniformity

The drug content in each formulation was determined by triturating 10 tablets and powder equivalent to average weight was added in 100ml of 0.1N hydrochloric acid, followed by stirring for 30 minutes. The solution was filtered through a 45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured using double beam UV spectrophotometer at 271nm using 0.1 N Hydrochloric acid as blank.

***In vitro* drug release studies**

Determination of theophylline release from different formulated tablets was performed using USP XXIV dissolution apparatus I at 100 rpm. The tablets were tested for drug release for 2 hours in 0.1 N HCl (900 mL) as the average gastric emptying time is about 2 hours. Then the dissolution medium was replaced with pH 7.4 Sorenson's phosphate buffer (900 mL) and tested for drug release for 3 hours as the average small intestine transit time is about 3 hours. At the end of the time periods, two samples each of 1 mL were taken, suitably diluted and analyzed for theophylline content at 271 nm using a double beam UV visible spectrophotometer.

The susceptibility of okra gum to the enzymatic action of colonic bacteria was assessed by reported method[24-26] where by drug release study was continuing in 100 mL of pH 6.8 phosphate buffered saline (PBS) containing 4% w/v of rat caecal contents with prior approval from institutional animal ethical committee (protocol no. IICP/PH/02-2010/03). The

caecal contents were obtained from male albino rats (weighing 150–200 g). Rats were killed before the commencement of drug release studies and abdomen were opened cecal contents immediately transferred in PBS to give a final caecal dilution of 4% w/v (previously bubbled with N_2). The drug release studies were carried out in USP dissolution rate test apparatus (apparatus 1, 100 rpm, 37°C) with slight modification. A beaker (capacity 150 mL) containing 100 mL of dissolution medium was immersed in the water contained in the 1000 mL vessel, which was, in turn, in the water bath of the apparatus. The tablets were placed in the baskets of the apparatus and immersed in the dissolution medium containing rat caecal contents. The experiment was carried out with continuous N_2 gas supply into the beakers to simulate anaerobic environment of the caecum. The drug release studies were carried out for 3 hours and 1 mL samples were taken at different time intervals without a prefilter and replaced with 1 mL of fresh PBS bubbled with N_2 gas. To the samples, 1 mL of methanol was added to ensure solubility of finely suspended drug particles released due to break down of okra gum by the caecal enzymes. The volume was made up to 10 mL with PBS, centrifuged and the supernatant was filtered through a bacteria-proof filter and the filtrate was analysed for theophylline content at 271 nm as described above. The above study was carried out on all formulations of theophylline (F1, F2 and F3) and also without caecal matter in pH 6.8 PBS (control).

Kinetics of analysis of dissolution data

To study the mechanism of drug release from the prepared matrices the release data were fitted to various mathematical models like First order, zero order, Hixon crowell, peppas and matrix type using software PCP Disso V 3.0[27-29].

***In vivo* evaluation**

Rabbits (New Zealand, White) of either sex weighing (2.5-3.0 Kg) were used to carryout the in vivo pharmacokinetic study with prior approval from the animal ethical committee of Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar (Protocol no. IICP/PH/12-201/03). According in vitro drug release study, formulation F3 showed minimum drug release in the upper GIT, hence selected for in vivo pharmacokinetic study and compared with in vivo pharmacokinetic parameters of theophylline marketed tablets preparation. The tablets containing 200mg theophylline were administered to rabbits with sufficient flush of water, in a group of three animals, in fasting conditions which was assisted by a local veterinary doctor. Food was withdrawn from the rabbits 12 hours before drug administration and until 12 hours post-dosing. All rabbits have free access to water throughout the study.

Blood samples were collected from marginal ear vein at defined time intervals. Blood collected was centrifuged at 5000 rpm for 15 minutes (Remi equipment, Mumbai, India) and drug concentration after deproteinization with acetonitrile was determined by HPLC assay (LC-20AT Prominence Liquid Chromatography, Shimadzu, Japan).

HPLC assay

The quantitative determination of drug in plasma was performed by HPLC assay using Methanol and Water (30:70, v/v) mixture as mobile phase delivered at 1.2 mL/ min by C_{18} (ODS) column. The column eluant was monitored at 271 nm using UV detector (SPD- 20A Prominence, Shimadzu).

Stability studies

To assess the long-term stability (2 years), formulation (F3) was stored at 40^0 C/75% RH for 3 months. At the end of study formulation was evaluated for physical appearance, color, drug content and drug release study. Drug release studies in rat caecal content medium were also carried out on sennosides matrix tablets of batch G3 after storage at 45°/75% RH for 3 months[30].

RESULTS AND DISCUSSION

The aim of the present study was to identify a suitable okra gum based matrix tablets for theophylline with sufficient mechanical strength and promising mouth-to-colon release profile. As a part of preformulation study, pure drug and the formulation were subjected to DSC studies. This study was carried out to establish that the therapeutically active drug has not undergone any changes. After spectral comparison it was confirmed that no incompatibility reactions takes place between drug and excipients. (DSC graphs may be provided on request).

Preliminary matrix tablets were prepared with 1:1 drug to polymer ratio using 2.5 % PVPK30 as binder to check the integrity of tablets in upper GIT by performing preliminary intact study. It was found that tablet remained intact in the physiological environment of stomach and small intestine for 5 h with a minimal release of ~25 %. Hence in present investigation tablets were prepared with higher concentration of okra gum as well as PVPK30 to increase the density of polymer in the formulation and to improve the biding of matrices, respectively, so as to minimize the further drug release in upper GIT. Three formulations were prepared where in okra gum was used as polymer by wet granulation method and its granulation and tableting properties with respect to colon targeting were evaluated. The granules of matrix tablet were prepared and characterized with respect to angle of repose, bulk density, tapped density, Carr's index, Hausner's ratio and drug content (Table 2). Angle of repose was less than 30^0 for all the batches of granules indicating satisfactory flow behavior. Rest of the parameters was also found within acceptable range.

The tablets of all batches were subjected to various in vitro evaluation tests like weight variation (10 tablets), friability (10 tablets), hardness (5 tablets) and content uniformity (10 tablets) and each test was repeated three times except hardness test. The weight variation and friability was less than 4% and 0.65%, respectively. Good uniformity in drug content was found among different batches of tablets and the drug content was more than 95 % (Table 2).

The ability of okra gum matrix tablet of theophylline to remain intact in the physiological environment of stomach and small intestine was assessed by conducting drug release studies under condition mimicking mouth to colon transit. *In vitro* dissolution studies for eight hours were performed as per the procedure described in methodology section. The F1, F2 and F3 formulations released 49.7 ± 2.5, 31.7 ± 3.4 and 27.7 ± 3.1% of theophylline, respectively, at the end of 5 hours dissolution study. Formulation F3 showed minimal amount of the drug released in upper GIT, this indicates satisfactory control over drug release in the physiological environment of stomach and small intestine. At the end of 2 hours and 5 hours of dissolution study photographs (Figure 1) of each batches were taken to check the integrity of prepared tablets visually.

Photographs showed that there was more erosion of tablets prepared with low concentration of okra gum (F1) and as concentration of okra gum increased the erosion was relatively lesser (F3). This attributed to increased density of the gum matrix at higher concentrations result in an increased diffusional path length and importantly presence of less population of microbes in the upper GIT that are generally responsible for the degradation of natural polysaccharides. On exposure to dissolution fluids, the gum becomes hydrated and forms a viscous gel layer that slows down further seeping-in of dissolution fluids towards the inner core. On coming into contact with biological fluids, okra gum swells up and the drug release takes place by diffusion. Mechanical erosion of the swollen okra gum layer follows. Unless the swollen gum layer erodes, further hydration and swelling of the okra gum does not take place. These might be possible reason for minimal drug release in upper GIT for the tablets prepared with higher amount of okra gum (F3). Drug releases from matrix tablets were by drug dissolution, drug diffusion or a combination of both.

After completing dissolution study in 0.1 M HCl (2h) and pH 7.4 Sorenson's phosphate buffer (3h), the dissolution study was continued both in simulated colonic fluid (rat cecal contents) and pH 6.8 PBS (control study) for another 3 h. At the end of 8h, the percentage drug releases for the formulations F1, F2 and F3 were found to be 100 ± 1.2, 88.8 ± 3.4 and 88.32 ± 1.3 in dissolution medium with rat cecal contents and 70.5 ± 2.4, 56.7 ± 0.9, and 51.8 ± 0.9 in dissolution medium without rat cecal contents (Figure 2).

The Student *t* test was used to find the statistical significance among the drug release from the matrices (n=3) in presence or absence of rat cecal contents. A value of *P* less than 0.05 was considered statistically significant. This difference was found to be statistically significant ($P<0.05$). The study showed that the release of drug in physiological environment of colon is due to the microbial degradation of okra gum in presence of rat cecal contents. On reaching colonic environment, the swollen gum layer would be acted upon by the colonic bacterial enzymes and release the drug contained in the swollen okra gum layer[31]. The dissolution study was conducted without rat cecal contents (control study) to ensure that the drug release was not due to the mechanical erosion which is likely to occur because of bowel moments in humans. In comparison to all formulations, F3 was found to control the drug release in upper GIT and gave abrupt release on reaching colonic fluid and found to have potential for colon specificity. Hence further studies like *In vivo* pharmacokinetic study in rabbit and stability study were performed using this formulation.

In order to gain insight into the drug release mechanism from the tablets, release data of selected tablets were examined according to the zero-order, first-order, matrix type, Hixson and Crowell type, Korsmeyer and Peppas type release models using software PCP DISSO V 3.0. These mathematical models are useful when attempting to elucidate and understand the mechanism of drug release from matrix formulations and their use is well documented[32-35]. When dissolution data are fitted to these empirical models, a high R^2 value indicates the appropriateness of the model to describing the possible mechanism of drug release. According to PCP DISSO V 3.0, the absorbencies at respective time intervals for all formulations were fed up in software with all details regarding conditions and in doing this software gave respective R^2 values for respective models. In present investigation it was found that formulations F1 and F2 follows zero order release kinetics where as F3 (Figure 3) follows Korsmeyer and Peppas model of release kinetics (Table 3). Applicability of peppas model indicates a change in surface area and diameter of tablets with the progressive dissolution of matrix as a function of time.

In vivo pharmacokinetic study was performed in White New Zealand rabbits and parameters like C_{max}, maximum concentration and T_{max}, time to reach maximum concentration are the values directly obtained from the plasma concentration time curve. A minimum drug release (10.14 ± 3.12 mcg/mL) was found for first three hours which might be attributed to drug that was presented on the surface of the tablets. Time taken to release maximum amount of drug was found to be 4.5 ± 0.2 hours with peak plasma concentration of 22.45 ± 4.12 mcg/mL. The higher concentration was also maintained for a longer period of time before dropping down (Figure 4).

Thus results showed that okra gum have a capacity of preventing the drug release in the stomach and intestine.

In view of the potential utility of F3 formulation for colonic release of theophylline, stability studies were carried out by storing the formulation at 40^0 C/75% RH for 3 months to access the long term (2 years) stability. There was no change in the physical properties of F3 formulation at the end of storage period. When the dissolution study was conducted in simulated GI and colonic fluids as described above, no significant

difference (P>0.05) was observed in the cumulative percentage of theophylline released from F3 stored at 40^0 C/75% RH for 3 months when compared to that released from same formulation before storage (Table 4). The insignificant changes in the physical appearance, drug content and dissolution profile of F3 formulation after storage at 40^0 C/75% RH for 3 months indicate that the formulation could have a minimum shelf life of 2 years[36].

Table 1: Composition of matrix tablets of theophylline

BATCH	FORMULATION INGREDIENTS				
	THEOPHYLLINE (mg)	OKRA GUM (mg)	PVPK30 (mg)	IPA (mL)	MAGNESIUM STERATE (%)
F1	200	200	16	qs	1
F2	200	300	20	qs	1
F3	200	400	24	qs	1

Table 2: Characterization of granules and matrix tablets of theophylline

PARAMETERS	F1	F2	F3
Granules			
Angle of repose (^{0})	27 ± 1.1	28 ± 0.8	28 ± 0.9
Bulk density	0.64 ± 0.1	0.54 ± 0.11	0.4 ± 0.09
Tapped density	0.88 ± 0.04	0.67 ± 0.09	0.56 ± 0.08
Compressibility index	27.273	19.403	28.571
Hausner's ratio	1.375	1.2407	1.4
Drug content (%)	99.45 ± 3.3	99.62 ± 3.1	99.35 ± 2.2
Tablets			
Weight variation (%)	± 1.2	± 1.9	± 1.5
Friability (%)	0.61	0.64	0.64
Hardness (kg/cm^2)	5.11 ± 0.1	5.46 ± 0.2	5.54 ± 0.3
Content uniformity (%)	98.94 ± 4.3	101.11 ± 3.5	102.10 ± 4.3
Rel_{5h} (%)	45	31	27

Table 3: Release kinetics analysis

MODELS	R SQUARE		
	F1	F2	F3
Zero order	0.9779	0.9596	0.9377
First order	0.9264	0.9096	0.9274
Matrix	0.9216	0.8489	0.8113
Korsmeyer and Peppas	0.9678	0.9524	0.9452
Hixon and Crowell	0.9345	0.9466	0.9275
Best fitting model	Zero order	Zero order	Korsmeyer and Peppas

Table 4: Drug release study for stability study

TIME HOUR	CUMULATIVE PERCENTAGE RELEASE (F3)	
	Before storage	After storage
0	0	0
1	8.84 ± 1.2	9.45 ± 1.1
2	12.89 ± 0.2	14.54 ± 0.3
3	21.71 ± 1.2	22.12 ± 1.2

4	23.53 ± 0.3	25.41 ± 0.9
5	28.52 ± 0.6	30.12 ± 0.8
6	37.52 ± 1.1	38.45 ± 1.1
7	60.82 ± 1.2	63.12 ± 0.6
8	88.32 ± 1.3	90.14 ± 1.0

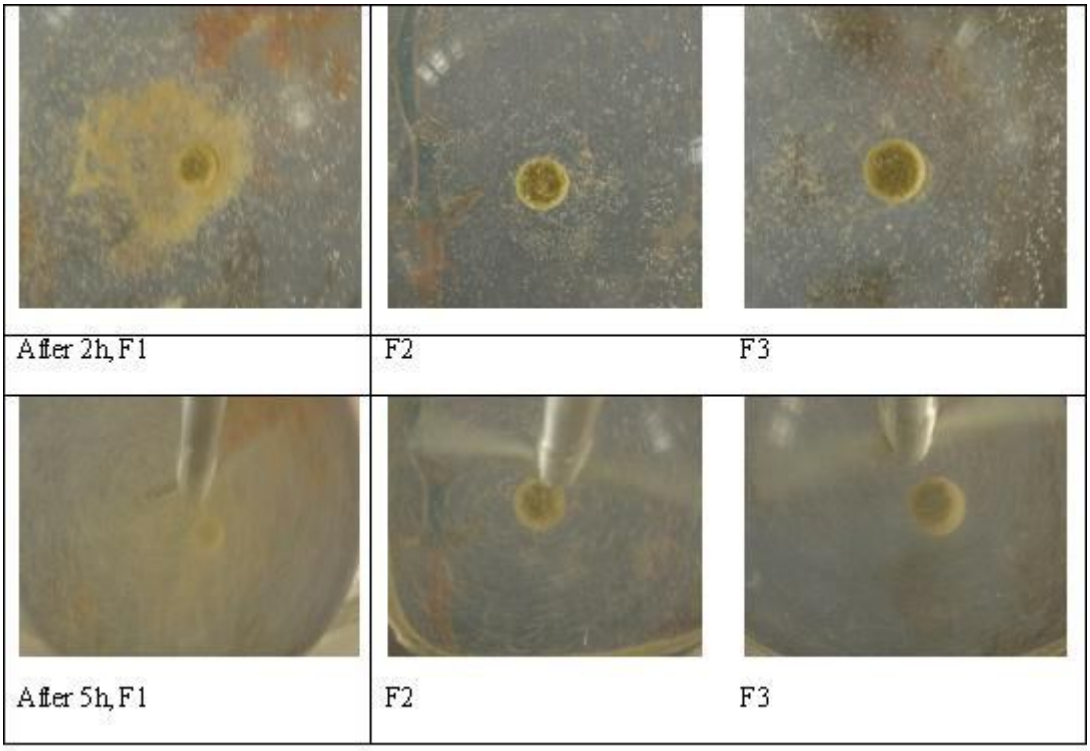

Fig. 1: Photographs of formulation (F1, F2 AND F3) after dissolution study 5 hours

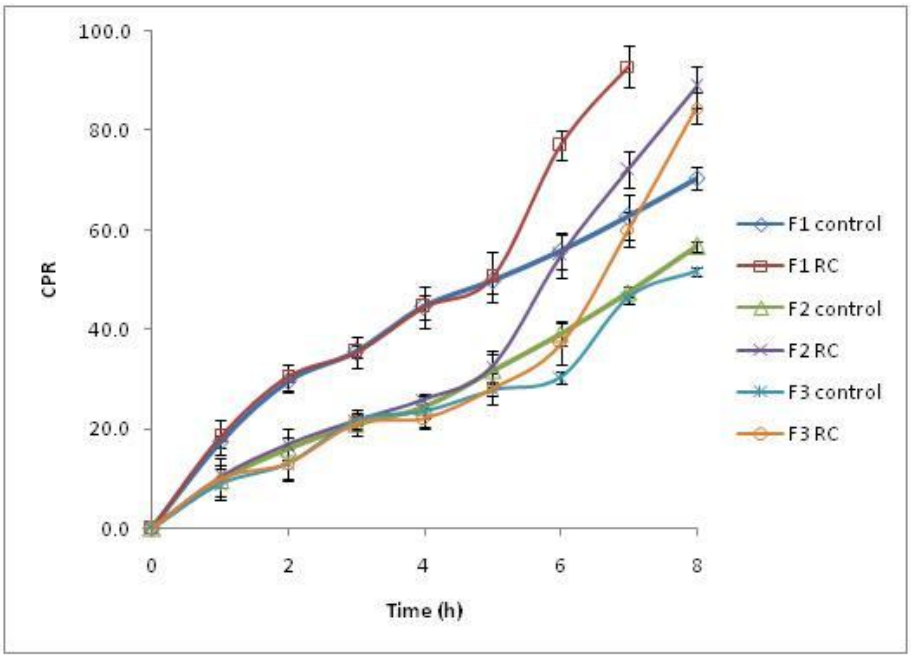

Fig. 2: In Vitro Dissolution Studies

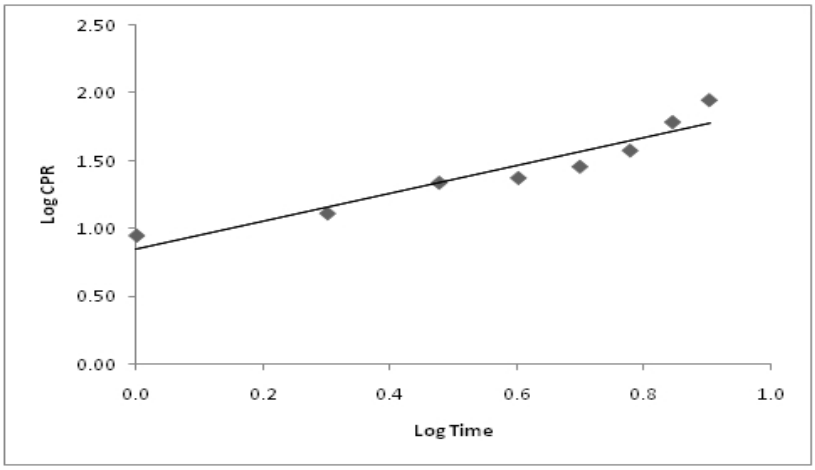

Fig. 3: Korsmeyer plot of drug release from formulation F3

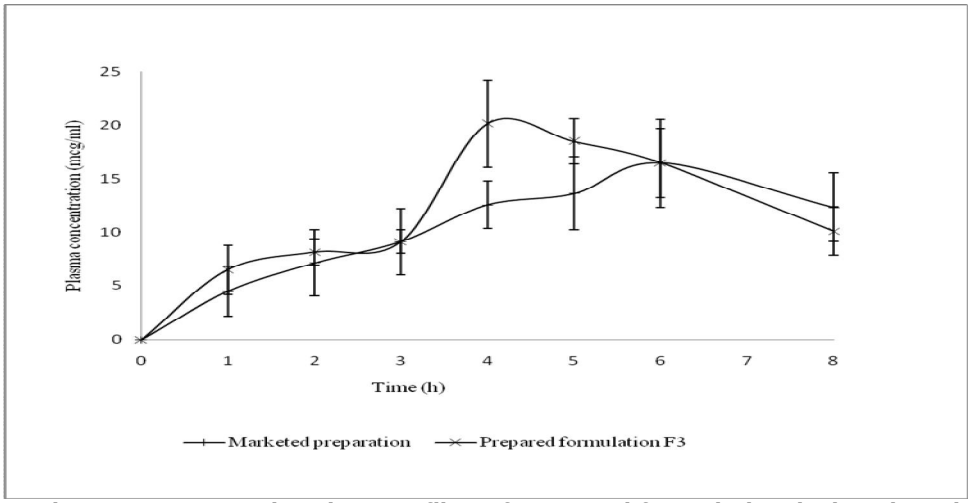

Fig. 4: Plasma concentration time profiles of prepared formulation (F3) and marketed formulation. Data are represented as mean ± SD (N=3)

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REFERENCES

1. D'Alonza GE, Smolensky MH, Feldman S, Gianotti LA, Emerson MB, Staudinger H and Steinijans VH. Twenty-four hour lung function in adult patients with asthma. Chronoptimized theophylline therapy once-daily dosing in the evening versus conventional twice-daily dosing. Amer Rev Respir Dis. 1990;142:84-90.
2. Nitin S, Sanjula B, Alka A and Javed A. Site Specific Chronotherapeutic Drug Delivery Systems: A Patent Review. Recent Patents on Drug Delivery & Formulation. 2009;3:64-70.
3. McConville JT, Ross AC, Chambers AR, Smith A, Florence AJ and Stevens NE. The effect of wet granulation on the erosion behaviour of an HPMC-lactose tablet, used as a rate-controlling component in a pulsatile drug delivery capsule formulation. Eur J Pharm Biopharm. 2004;57:541-549.
4. Kumar P and Mishra B. Colon Targeted Drug Delivery Systems - An Overview. Curr Drug Del. 2008;5:186-198.
5. Al-Saidan SM, Krishnaiah YSR, Satyanarayana S and Rao GS. In Vitro and In Vivo Evaluation of Guar Gum-Based Matrix Tablets of Rofecoxib for Colonic Drug Delivery. Curr Drug Del. 2005;2: 155-163.
6. Krishnaiah YSR, Satyanarayana V, Dinesh B and Karthikeyan RS. In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil. Eur J Pharm Sci. 2002;16:185-192.
7. Sinha VR and Rachna K. Polysaccharides in colon-specific drug delivery. Int J Pharm. 2001;224:19-38.
8. Kinget R, Kalala K, Vervoort L and Van den MJ. Colonic drug targeting. J Drug Target. 1998;6:129-149.
9. Anil KP and Betty P. Colon Targeted Drug Delivery Systems: A Review on Primary and Novel Approaches. OMJ. 2010;25:70-78.
10. Ofoefule SI and Chukwu A. Application of Abelmoschus esculentus gum has been used as mini matrix for Furosemide and

diclofenac sodium tablets. Indian J Pharm Sci. 2001;68:532-35.
11. Ofoefule SI, Chukwu A and Anyakoha N. Application of Abelmoschus esculentus in Solid Dosage Formulation 1: Use as a Binder for a poorly water soluble drug. Indian J Pharm Sci. 2001;63:234-238.
12. Attama AA, Adikwu MU and Amorha CJ. Release of indomethacin from bioadhesive tablets containing carbopol 941 modified with Abelmuschus esculentus (okra) gum. Boll Chim Farm. 2003;142:298-302.
13. Kalu VD, Odeniyi MA and Jaiyeoba KT. Matrix Properties of a New Plant Gum in Controlled Drug Delivery. Arch Pharm Res. 2007;30:884-889.
14. Mishra A, Clark JH and Pal S. Modification of Okra mucilage with acrylamide: Synthesis, characterization and swelling behavior. Carbohydrate Poly. 2008;72:608-615.
15. Ndjouenkeu R, Goycoolea FM and Morris ER. Rheology of okra and dika nut polysaccharides. Carbohydrate Poly. 1996;29:263-269.
16. Sweetman SC. Martindale, the Complete Drug Reference. 33rd Edn. London, U.K Pharmaceutical Press; 2002.
17. Satyanarayan S and Krishna B. Colon specific drug delivery. In: Jain NK, Editor. Advances in novel and controlled drug delivery, New Delhi; CBS Publications and distributors; 2003:347-388.
18. Patel MM and Amin AF. Design and optimization of colon-targeted system of theophylline for chronotherapy of nocturnal asthma. J Pharm Sci. 2011;100(5):1760–1772.
19. Gilbert SB and Neil RA. Tablets. In: Leon L, Herbert AL, Joseph LK, Editors. The Theory and Practice of Industrial Pharmacy, (3rd ed.), Publishing House; Bombay; 1990:293-345.
20. Fatmanur TD, Sevgi T and Boyuna OK. In-vitro and In-vivo Evaluation of Mesalazine-Guar Gum Matrix Tablets for Colonic Drug Delivery. J Drug Target. 2004;12:105-112.
21. Government of India, In: Indian Pharmacopoeia; Ministry of Health and Family Welfare, Published by the The Controller of Publications, Delhi, Vol. I, 1996:177-188.
22. Oyi AR, Shittu AO and Mahmud AS. Physicochemical Characterization of Acacia Sieberiania Gum Characterization of tablets. Indian J Novel Drug del. 2010;2:99-102.
23. Onunkwo GC. Evaluation of okro gum as a binder in the formulation of thiamine hydrochloride granules and tablets. Res In Pharm Biotech. 2010;2:33-39.
24. Chorasia MK and Jain SK. Potential of guar gum microspheres for target specific drug release to colon. J Drug Target. 2001;2:435-442.
25. Fukui E, Miyamura N, Uemura K and Kobayashi M. Preparation of enteric coated timed-release press-coated tablets and evaluation of their function by in vitro and in vivo tests for colon targeting. Int J Pharm. 2000;204:7-15.
26. Chourasia MK and Jain SK. Pharmaceutical approaches to colon targeted drug delivery system. J Pharm Sci. 2003;6:33-66.
27. Paradkar A, Maheshwari M, Tyagi AK, Chauhan B and Kadam SS. Preparation and Characterization of Flurbiprofen Beads by Melt Solidification Technique. AAPS PharmSciTech. 2003;4: article 65.
28. Anagha J, Shivajirao SK and Aatmaram PP. Technical Note: The Effect of Alcoholic Beverages on Sustained Release. Pharm Tech. 2010;34:47-49.

29. Purushothaman M and Vijaya R. Formulation optimization and release kinetics of Metronidazole matrix, compression and spray coated tablets: Effect of organic acid on colon targeted drug delivery system. Int J Res Pharm Sci. 2010;1:551-562.
30. Carstesen JT. Drug Stability: Principle and Practices. 2nd ed. New York: Marcel Dekker; 1995.
31. Rama Prasad YV, Krishnaiah YSR and Satyanarayana S. In vitro evaluation of guar gum as a carrier for colon-specific drug delivery. J Control Rel. 2010;51: 281-287.
32. Syed NHS, Sajid S, Muhammad AC, Muhammad SHA, Nisar-ur R and Sattar B. Formulation and evaluation of natural gum-based sustained release matrix tablets of flurbiprofen using response surface methodology. Drug Dev Ind Pharm. 2009;35:1470-1478.
33. Vijayalakshmi P, Kusum Devi V, Narendra C and Srinagesh S. Development of Extended Zero-Order Release Gliclazide Tablets by Central Composite Design. Drug Dev Ind Pharm. 2008;34:33–45.
34. Ashmitha P, Munasur VP, Yahya E, Choonara IM and Thirumala G. Comparing the Mucoadhesivity and Drug Release Mechanisms of Various Polymer-Containing Propranolol Buccal Tablets. Drug Dev Ind Pharm. 2008;34:189–198.
35. Lutfi G and Esmaeil J. Preparation and In Vitro Evaluation of Controlled Release Hydrophilic Matrix Tablets of Ketorolac Tromethamine Using Factorial Design. Drug Dev Ind Pharm. 2008;34:903–910.
36. Mathews BR. Regulatory aspects of stability testing in Europe. Drug Dev Ind Pharm. 1999;25:831-856.