CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.159276

Available online at: http://www.iajps.com Research Article

FORMULATION AND *IN-VITRO* EVALUATION OF A NOVEL BIODEGRADABLE POLYMER BASED MICROPARTICULATE SYSTEM FOR POTENTIAL COLON TARGETED DRUG DELIVERY

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Abstract:

The objective of present study is to design a novel multiparticulate system for colon targeting drug delivery system of cyclophosphamide (CPM) using biodegradable Konjac glucomannan (KGM) as a carrier for colorectal cancer treatment. Glucomannan is a high-molecular weight, water-soluble, non-ionic polysaccharide extracted from the tuber or root of the elephant yam, also known as konjac (Amorphophallus konjac or Amorphophallus rivieri). cyclophosphamide (CPM) has been the only agent with clinically active against colorectal cancer. Different batches of CPM granules were prepared and coated with KGM. Optimized CPM granules (KGM-STMP) were evaluated for flow properties, granular parameters, weight variation and content uniformity. The prepared formulations were subjected for in-vitro drug release studies in simulated gastric and intestinal fluids. It was found that during 2 hr at pH 1.2 HCl (SGF), 3 hr at pH 7.4 phosphate buffer (SIF) only less than 20% of drug was released. The drug release studies was also carried out in simulated colonic fluid (SCF) of pH 6.8 containing β-mannanase in order to mimic the conditions from mouth to colon. Drug release from 5-FU granules coated with KGM-STMP followed diffusion dependent zero order kinetics. Further, report suggested that konjac glucomannan (KGM) was biodegradable and susceptible to the colonic microfloras under anaerobic environments. Spectroscopic, (FTIR), X-ray Diffraction (X-RD) and DSC studies concluded that no polymer-drug interaction was concluded. Accelerated stability studies indicated that no significant changes, in drug release and micrometric patterns for observed when stored at 40± 2ºC / 75±5% RH. Therefore, it was concluded that konjac glucomannan (KGM) is a promising potential carrier for targeting CPM in the vicinity of colon in order to treat colon cancer effectively.

Key words: *Cyclophosphamide (CPM), Konjac glucomannan (KGM), multiparticulate system, colon targeted.*

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Please cite this article in press as Madhu G and Ramarao N, ***Formulation and In-Vitro Evaluation of A Novel Biodegradable Polymer Based Microparticulate System for Potential Colon Targeted Drug Delivery****, Indo Am. J. P. Sci, 2016; 3(9).*

INTRODUCTION:

The colon targeted drug delivery is beneficial for the localized treatment of several colonic diseases mainly inflammatory bowel diseases (IBD), irritable bowel syndrome and colon cancer [1-3]. To achieve clinically relevant bioavailability of poorly absorbed drugs from the upper parts of the gastrointestinal tract because of their polar nature and/or vulnerability to chemical and enzymatic degradation in the small intestine specifically for proteins and peptides [4-7].

Konjac glucomannan is a high-molecular weight, water-soluble, non-ionic polysaccharide extracted from the tuber or root of the elephant yam, also known as *konjac* (*Amorphophallus konjac* or *Amorphophallus rivieri*)[8]. KGM is a polysaccharide of the mannan family, very abundant in nature, specifically in softwoods (hemicellulose), roots, tubers and many plant bulbs. Despite the variety of sources, the most commonly used type of glucomannan (GM) is named *konjac* GM (Fig.1), which is extracted from tubers of Amorphophallus *konjac*. Irrespective of its origin, KGM is composed of β-1, 4 linked D-mannose and D-glucose monomers [9].

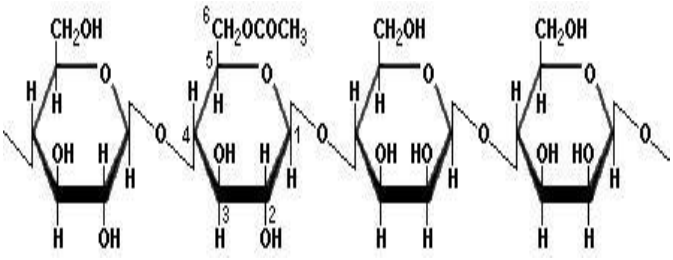

Fig 1: Structure of *Konjac* Glucomannan

Targeted drug delivery may provide maximum therapeutic activity by preventing degradation or inactivation of drug during transit to the target site. An ideal targeted delivery system should be nontoxic, biocompatible, and biodegradable and physicochemical stable *in-vitro* and *in-vivo*. The targeted drug delivery is dependent on the identification and exploitation of attribute that is specific to the target organ [10-17].

MATERIALS AND METHODS:

Materials

Cyclophosphamide was obtained as a gift sample from Biophore Pharmaceutical Pvt. Ltd, (Hyderabad, India). ***Konjac*** glucomannan was purchased from Dalian Ruishengda International Trade Co., Ltd., Western Hills Village Shahekou District Dalian, China. Sodium trimetaphosphate (STMP)was purchased from Central Drug House, Mumbai. Magnesium stearate, Lactulose, Talc were purchased from Merck Chemicals Corporation Mumbai, India. All other chemicals and solvents are of reagent grade. Double distilled water (DDW) was prepared using the in-house distillation unit.

Preparation of cyclophosphamide granules[18-20]:

Different batches of matrix granules of cyclophosphamide were formulated by wet granulation method. All ingredients were weighed on digital weighing balance and passed through sieve no. 40. The sieved ingredients (except lubricants and glidants) were placed in mortar and pestle for mixing. Then distilled water was added for wet massing, to form a coherent mass. The obtained coherent mass was placed on the sieve no. 10 and forced out through a sieve screen where it was continuously formed into extradites. Cylindrical shaped extradites were then transferred and spreaded out on perforated trays for drying of product in hot air oven at 50 ^{0}C ± 2 ^{0}C for 1.5-2 hours. Then dried cylindrical mass was forcefully passed through sieve no. 22 to get uniform sized granules. Lubricants (magnesium stearate) and glidant (talc) were added to improve flow properties. . Used ingredients are listed in table.1.

Table 1: Ingredients used in preparation of granules.

S.NO	Ingredients	Quantity (mg)
1	Cyclophosphamide	300
2	Lactose	785
3	Magnesium stearate	50
4	Talc	600

Crosslinking of *Konjac* glucomannan[21-25]:

According to the methodology used by Gliko-Kabir et al.[26-28] ***konjac*** glucomannan dispersion was prepared. The dispersion was stirred for 2 h to allow the polymer to completely dissolve. The following amounts of sodium trimetaphosphate (STMP) were added to three 100 mL portions of the dispersion: 5 mL 30% to the first (sample A), 10 mL 30% to the second (sample B), 15 mL 30% to the third (sample C), 20 mL 30% to the fourth (sample D) and 30 mL 30% to the fifth (sample D) . Five hundred milligrams of drug loaded granules were coated in fluidized bed coater by different coating formulations. Coating conditions are listed in table 2.

Table 2: Coating parameter

Inlet temp. (^{0}C)	Exhaust temp. (^{0}C)	Atomization (bar)	Spray rate (g/min)	Pan rate (rpm)	Weight gain (%)	Curing (mins)
40-45	32-35	2.0 – 2.5	2 – 4	9-11	3%	30

Physical characterization of granules:
All physical tests of granules were performed like Bulk density, Tapped density, Compressibility Index, Hausner's ratio and Loss on drying, PSD by sieve analysis.

Drug content determination:
The test was performed with formulations CTDD-1 to 5 by assaying them individually according to USP limits. The capsule was crushed and dissolved in phosphate buffer saline solution (pH 7.4) and volume made up to 100 ml in the volumetric flask. A 0.1 ml aliquot was taken out and volume made up to 10 ml with PBS (pH 7.4) solution and filtered through Whatman No.1 filter paper.

RESULTS AND DISCUSSION:

The granules of all the formulations were evaluated for angle of repose, bulk density, tapped density, compressibility index and hausner's ratio. The angle of repose was found to be 31°51' ±0.77–33°69'±0.74. It indicates that granules have a good flow property. The bulk density and tapped density was found to be in the range of 0.36±0.00-0.4±0.01 gm/cc and 0.39±0.0-0.48±0.02gm/cc respectively. The compressibility and hausner ratio was found to be 7.69±1.86 to 15.50±1.74 and 1.08±0.04 to 1.17±0.04 indicating good flow character of the granules (table-3). All the results are within the prescribed limit [29-31].

Prepared colon targeted drug capsule formulations were subjected to preliminary *in-vitro* release studies. Dissolution was carried in three media, namely simulated gastric fluid (acidic buffer, pH 1.2) for the first two hours, simulated intestinal fluid (sorensen's phosphate buffer, pH 6.8) for three hours and simulated colonic fluid (phosphate buffer pH 7.4) evaluated in presence of β-mannanase for the subsequent hours[32-36]. After ingestion of the capsule, there was no drug release in the stomach due to the acid resistibility of the polymeric layer with all the formulation, indicating the efficiency of glucomannan as biodegradable enteric coating polymer. This polymer proved that the drug was not released in the stomach. The release profile of the various formulations coated with KGM crosslinked with STMP is given in table 4 and figure 2 respectively. The drug release was slow in first 5 hours followed by spread over 24 hour and depends upon concentration of phosphated glucomannan [37-41].

When FT-IR and DSC studies were performed, it was found that there was no interaction between CP and polymers used (fig. 3and 4). The physicochemical properties of the entire KGM coated pellets batches were found to be in limits. The optimized KGM-STMP coated pellets-filled-capsule formulation CTDD-4 releases CP in presence of β-mannanase after a lag time of 98.75% at the end of 24 hrs respectively.

Table 3: Evaluation of matrix granules of CP

Formulation Code	Bulk density*(g/cc)	Tapped density*(g/cc)	Carr's index* (%)	Hausner's ratio*	Angle of repose*(Θ)	Drug content*(%)
G1	0.41±0.01	0.48± 0.02	15.50±1.74	1.17±0.04	$33^0$14'±0.66	98.20± 1.33
G2	0.40±0.01	0.46±0.02	13.04±1.66	1.15±0.02	$33^0$69'±0.74	100.87 ± 1.31
G3	0.38±0.05	0.42±0.01	9.52±1.27	1.10±0.02	$30^0$30'±0.67	101.2 ± 1.89
G4	0.36±0.00	0.39±0.01	7.69±1.86	1.08±0.04	$32^0$42'±0.59	95.99 ± 1.96
G5	0.36±0.01	0.40±0.01	10±2.01	1.11±0.03	$31^0$51'±0.77	97.04 ± 2.13

Table 4: *In-vitro* dissolution studies of CTDD filled with granules coating with different ratios of KGM-STMP solution

S.NO	Time (in hrs)	Formulation Code				
		CTDD-1	CTDD-2	CTDD-3	CTDD-4	CTDD-5
1	2	1.31±1.06	0.86±1.27	1.22±0.41	1.49±0.23	1.53±0.35
2	5	11.65±1.59	12.18±1.87	11.05±1.56	12.14±2.31	11.75±1.22
3	7	28.42±1.34	31.69±3.56	30.48±0.16	31.72±1.69	29.43±1.28
4	9	32.54±2.79	45.04±1.67	43.25±2.35	44.33±3.78	33.27±1.04
5	12	41.50±3.45	55.29±2.59	53.69±1.24	61.29±1.45	40.37±2.11
6	15	49.28±2.54	59.18±3.65	57.46±1.36	69.78±4.23	48.53±1.05
7	18	55.29±0.49	62.01±1.16	61.55±1.14	78.26±2.38	54.11±1.34
8	21	68.37±3.52	71.55±2.92	70.24±0.33	89.98±0.46	67.42±1.34
9	24	82.86±1.64	87.46±2.06	85.13±2.41	98.75±1.33	82.47±2.54

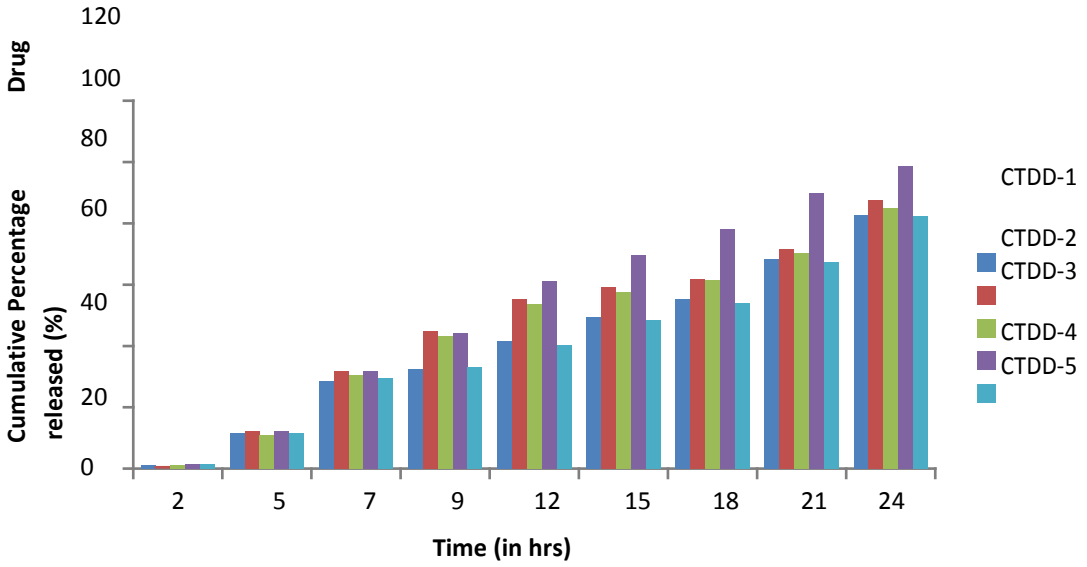

Fig 2: *In–vitro* release study of capsule coated with different concentrations of phosphated glucomannan

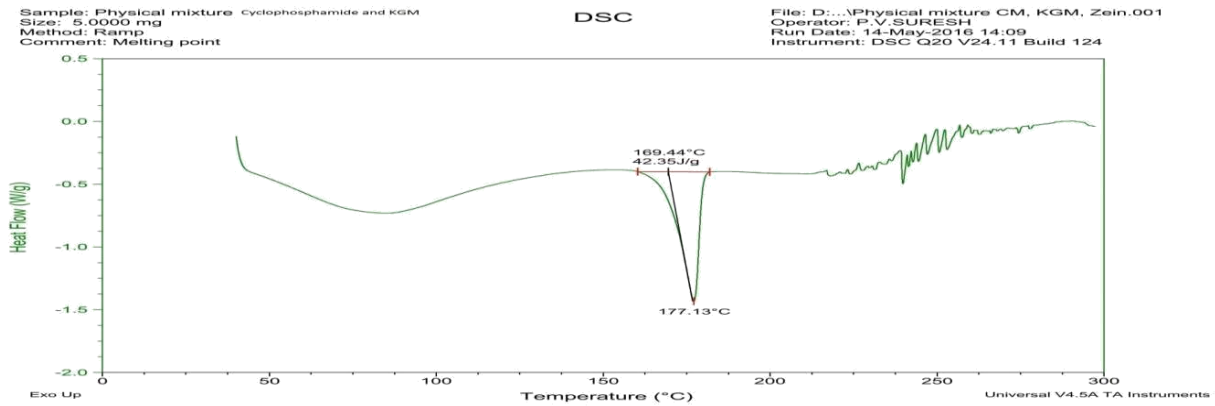

Fig 3: DSC thermogram of Physical mixture of Cyclophosphamide and *Konjac* Glucomannan

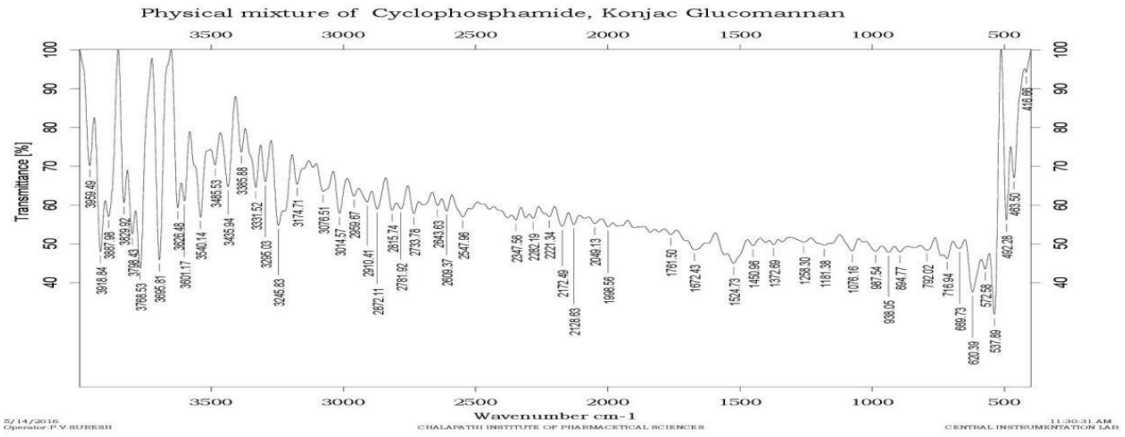

Fig 4: FT-IR graph of physical mixture of Cyclophosphamide and *Konjac* Glucomannan

CONCLUSION:

In-vitro release of cyclophosphamide (CPM) granules was evaluated in presence of β-mannanase. It was concluded that the reservoir-like drug release system of phosphated KGM membrane could be potencial as an colon-specific drug delivery. Therefore, it was concluded that *konjac* glucomannan (KGM) is a promising potential carrier for targeting CPM in the vicinity of colon in order to treat colon cancer effectively. The results of our study clearly indicate that there is great potential in delivery of cyclophosphamide to the colonic region as an alternative to the conventional multiparticulate system.

AKNOWLEDGEMENT:

This work was supported by Research grant (Endt. No F1-17.1/2012-13/UGC-2012-13-SC-AND-34273) from University Grants Commission (UGC), New Delhi, India. The author also sincerely thankful to Prof. Rama Rao Nadendla sir, Principal, Chalapathi Institute of Pharmaceutical Sciences (CIPS) and management of Chalapathi Institutions, Guntur, Andhra Pradesh for providing the necessary facilities to carry out this research work.

REFERENCES:

1.Zahirul M. I. Khan, Zeljiko Prebes, Nevenka Kurjakovic, "A pH- dependent colon targeted oral drug delivery system using methacrylic acid copolymers". I Manipulation of drug release using Eudragit ® L100-55 and Eudragit® S100 combinations. Journal of controlled release, 1998; 58: 215-222.

2.Sinha V.R. Rachna Kumaria, "Microbially triggered drug Delivery to the colon", European Journal of Pharmaceutical Sciences, 2003; 18:3-18.

3.Mrsoy Randall. J., " The colon as a site for drug delivery"., Journal of controlled Release, 1992; 22:15-34.

4.Krishnaiah Y.S.R., Veer Raju P., Dinesh Kumar B., Satyanarayana v., Karthikeyan R.S. Bhaskar P. "Pharmacokinetic evaluation of guar gum based colon targeted drug delivery systems of mebendazole in healthy volunteers"., Journal of controlled release, 2003; 88:95-103.

5.Eiji Fukul Nobuteru Miyamura, Katsuji Uemura, Masao Kobayashi., "Preparation of enteric coated timed –release press coated tablets and evaluation of their function by in-vitro and in–vivo tests for colon targeting". International Journal of Phamaceutics, 2000; 204:7-15.

6.Christina Graffiner, Severree A, Sande, Sayesh .F. Ahrabi, Grate Madsen, Knut Dyrstad, "Development of pectin matrix tablets for colonic delivery of model drug ropivacaine". European Journal of Pharmaceutical Sciences, 2000; 10 43-52.

7.Singh BN. Modified release solid formulations for colonic delivery. Recent Patents Drug Delivery Formulations. 2007; l: 53-63.

8.Alvarez-Mancenido F, Landin M, Martinez-Pacheco R. Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. Eur J Pharm Biopharm. 2008; 69:573-581.

9.Lehmann, K.O.R.; Dreher, K.D. Methacrylate-galactomannan coating for colon-specific drug delivery. Proc. Int. Symp. Control. Rel. Bioact. Mater., 1991; v.18, n.194: 331-332.

10.Mura P, Maestrelli F, Cirri M. Development of enteric-coated pectin-based matrix tablets for colonic delivery of theophylline. J. Drug Target. 2003; 11: 365-371.

11.Hiorth M, Versland T, Heikkila J. Immersion coating of pellets with calcium pectinate and chitosan. Int. J. Pharm. 2006; 308:25-32.

12.Rathod S: Colon Targeted Pulsatile Drug Delivery: A Review. Pharm Rev. 2007; 5(2).

13.Wu.B, Chen.Z, Wei.X, Sun.N, Lu.Y, Wu.W. Eur J Pharm Biopharm 2007; 67: 707–714.

14.Alvarez-Mancenido F, Landin M, Martinez-Pacheco R. Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. Eur J Pharm Biopharm. 2008; 69:573-581.

15.Rana Mazumder, et al., Formulation and in vitro evaluation of natural polymers based microspheres for colonic drug delivery. Int. J. Pharm. and Pharm. Sci., Vol 2, 2010.

16.Chien YW. Oral drug delivery and delivery systems. In: Chien YW, editor. Novel drug delivery systems. New York: Marcel Dekker Inc; 1992; 139-196.

17.Sharma Madhu1, Joshi Baibhav2, Bansal Monika1, Goswami Manish, Formulation and evaluation of colon targeted tablets of Mesalazine, Journal of Drug Delivery & Therapeutics; 2012; 2(5), 24-36

18.Patel MM, Shah TJ, Amin AF, Design, development and optimization of a novel time and pH dependent colon targeted drug delivery system, Pharmaceut Develop Tech, 2009;14(1), 65-72.

19.Gohel MC, Parikh RK, Parikh SA, Nagori, MR, Design of a potential colonic drug delivery system of mesalamine, Pharmaceut Develop Tech, 2008;13(5):447-456.

20.Watts PJ, Illum L, Colonic drug delivery, Drug Dev Ind Pharm, 1997; 23:893-913.

21.Sinha VR, Kumria R, Polysaccharides in colon-specific drug delivery, Int J Pharm, 2001; 224:19-38.

22.Chien, Y., Novel drug delivery systems. Marcel Dekker Inc: New York: 1992; 2nd Ed: 139-140.

23.Oosegi, T., Onishi, H., Machida, Y., International Journal of Pharmaceutics., 2008, 80-88.

24.uganesh Ramasamy1,4, Uma Devi Subbaih Kandhasami2,3,*, Himabindhu Ruttala4, Suresh Shanmugam, Formulation and evaluation of xanthan gum based aceclofenac tablets for colon targeted drug delivery , Brazilian Journal of Pharmaceutical Sciences, 2011; 47(2).

25.Akanksha, T.; Raj Kumar, S. Natural polymer in colon targeting. Int. J. Pharm. Clin. Res., 2009; 1(2): 43-46.
26.Chourasia, M.K.; Jain, S.K. Pharmaceutical approaches to colon targeted drug delivery systems. J. Pharm. Sci., v.6, n.1, p.33-36, 2003; 6(1): 3-36.
27.Gliko-Kabir, I.; Penhasi, A. & Rubinstein, A. - Carbohyd. Res., 316, p.6. (1999). http://dx.doi.org/10.1016/S0008-6215(99)00025-7
28.Gliko-Kabir, I.; Yagen, B.; Penhasi, A. & Rubinstein, A. J. - Control. Release, 63, p.121 (2000). http://dx.doi.org /10.1016/S0168-3659(99)00179-0
29.Gliko-Kabir, I; Yagen, B.; Penasi, A.; Rubinstein, A. - Pharmaceut. Res., 15, p.1019 (1998). PMid:9688054.
http://dx.doi.org/10.1023/A:1011921925745
30.Dhopeshwarkar, V.; O'keeffe, J.C.; Horton, M. Development of an oral sustained release antibiotic matrix tablet using invitro/invivo correlations. Drug Develop. Ind. Pharm., 1994; 20(11): 1851-1867.
31.Liu, L.; Fishman, M.L.; Kost, J.; Hick, K.B. Chondroitin sulphate based systems for colon – specific drug delivery via oral route. Biomaterials, 2003; 246(3): 3333-3343.
32.Momin, M.; Pundarikakshudu, K. In vitro studies on guar gum based formulations for the colon targeted delivery of sennosides. J. Pharm. Pharmaceut., 2004; 7(3): 325-331.
33.Mundargi, R.C.; Patil, S.A.; Agnihotri, S.A.; Aminabhavi, T.M. Development of polysaccharide-based colon targeted drug delivery systems for the treatment of amoebiasis. Drug Dev. Ind. Pharm., 2007; 33(3): 255-264.
34.Mutalik,S.; Manoj, K.; Sreenivasa Reddy, M.; Kushtagi, P.; Nayak Usha, A.; Anju, P.; Kumar Ranjith, A.; Udupa, N. Chitosan and enteric polymer based once daily sustained release tablets of Aceclofenac: in vitro and in vivo studies. AAPS PharmSciTech., 2007; 9(2): 651-659.
35.Paharia, A.; Yadav, A.K.; Rai, G.; Jain, S.K.; Pancholi, S.S.; Agrawal, G.P. Eudragit-coated chondroitin sulphate microspheres of 5-fluorouracil for colon targeting. AAPS PharmSciTech., 2007; 8(1): 135-146.
36.Madhu Gudipati, Ramarao Nadendla, Konjac glucomannan, a promising polysachharide for colon release: a review, Europan J. Biomed. Pharm. Sciec. 2016; 3(9): 514-526.
37.Sarasija, S.; Hota, A. Colon-specific drug delivery systems. Ind. J. Pharm. Sci., 2000; 62(1): 1-8.
38.Sinha, V.R.; Mittal, B.R.; Bhutani, K.K.; Rachna Kumari. Colonic drug delivery of 5 fluorouracil: an in vitro evaluation. Int. J. Pharm., 2004; 269(1): 101-108.
39.Watts, P.J.; Illum, L. Colonic drug delivery. Drug Dev. Ind. Pharm., 1997; 23(9): 893-913.
40.Wen H, Park K. Oral Controlled Release Formulation Design and Drug Delivery: Theory to Practice. Wiley publishers. 2011; 54(1): 118-119.
41.Priti Girotra, Shailendra Kumar Singh,Formulation Optimization for Colon Targeted Delivery of Katira Gum Matrix Tablets Containing Azathioprine, Int. J. Pharm. Sciec.D. Res. 2013; 5(4): 133-140.