ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Volume - 01 Issue - 06 NOV-DEC 2013

website: www.ajprd.com
editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

FORMULATION AND *IN-VITRO* EVALUATION OF FLOATING PULSATILE BEADS OF INDOMETHACIN FOR SITE AND TIME-SPECIFIC RELEASE

T.Sudhamani*, M.Radhakrishnan

Department of Pharmaceutics, The Erode College of Pharmacy and Research, **Erode, Tamil Nadu, India**

Received: 15 Nov. 2013 ***Revised and Accepted: 27 December 2013***

ABSTRACT

The objective of this work was to develop site and time specific oral floating pulsatile multiparticulate system of indomethacin intended for chronotherapy. This was achieved by formulation of beads with blend of floating and pulsatile principles showed typical two phase release profile with a lag time in acidic stomach pH correlated well with floating time followed by rapid release only in small intestine pH and which was designed by formulation of water insoluble complexes of calcium pectinate with drug. The formulated beads were evaluated for bulk density, particle size, percentage encapsulation, buoyancy and in-vitro release studies. The floating pulsatile beads obtained were decreased bulk density below one and provided higher percentage of encapsulation. All beads showed good buoyancy as 4 to 6 hours of lag time in stomach pH followed by rapid pulse release in small intestine pH made suite for site and time-specific pulsatile release of drug.

Key words: *calcium pectinate beads, indomethacin, low methoxy pectinate, rheumatoid arthritis, floating pulsatile drug delivery.*

INTRODUCTION

A site and time specific release pulsatile drug delivery system characterized by a lag time followed by rapid and complete drug release where and when the drug has greater absorption based on circadian variation of disease[1]. Various diseases like asthma, hypertension, arthritic show circadian variation, that demand time-scheduled drug release for effective drug action. Rheumatoid arthritis shows circadian variation in its symptoms of morning stiffness associated with pain at the time of awakening that demands time-scheduled drug release for effective drug action [2].

* For Correspondence:
Mrs.T.Sudhmani
Assistant Professor, Department of Pharmaceutics,
The Erode College of Pharmacy and Research,
Veppampalayam (po), **Erode, Tamil Nadu-638112.**
Mobile No: +91 9842457080
Email: tsmkrishv28@yahoo.com

A review of the chronobiology of rheumatoid arthritis highlighted that stiffness, swelling and pain of one or more joints of the body characteristically most severe in the morning. It has been reported that, with arthritis exhibit maximum stiffness and maximum functional disability at 6:00 AM and maximum pain at 8:00 AM. Grip strength has been found to reach minimum at 6:00-8:00AM. Therefore, there is a need of site and time specific drug release on rheumatoid arthritis therapy [3, 4].

The design of site and time specific drug delivery systems involves release of drug only at a specific site in the GIT at particular time only. This system, suit for the drugs having characters as i)destroyed in the stomach/ by intestinal enzymes, ii) known to cause gastric distress iii) absorbed from a specific intestinal site or iv) meant to exert local effect at a specific gastrointestinal site [5]. Numerous approaches to provide site and time specific drug release exists. Most of such delivery

systems are single unit devices having in common risk of losing their effects too early due to their all or nothing emptying from the GI tract. But the formulated multiparticulate systems have advantages over single unit ones as they can spread out in more uniform manner over a larger surface area in the GIT and also ensure more uniform drug absorption. Because there are light weight, small size and smaller dose variation due to their large member of particles [6-8].

Indomethacin is a low dose non-steroidal anti-inflammatory (first line) drug, being used successfully for the treatment of rheumatoid arthritis and other joint pains which is insoluble in gastric fluid (pH 1.2) and soluble in intestinal fluid of small intestine (pH 7.4) [9,10]. Indomethacin has greater absorption in morning as compared to evening and site specific absorption from small intestine [11]. Hence drug release at proper time and site of better absorption can improve therapeutic efficacy of drug. This is of more concern for site and time specific drug delivery that is meant for pulsed drug release after a lag period following oral administration of dosage form. It also avoids adverse reactions like gastric irritation, abdominal pain, active GI bleeding, nausea, vomiting etc. Hence Indomethacin was used as a model drug.

The main criteria for choosing the polymers was that they should be insoluble in gastric fluid (pH 1.2) and soluble in the fluid of small intestine and large intestine (pH 7.4), so that the beads remain undissolved in gastric fluid and when the beads reaches the small intestine, the polymer dissolves to release the drug at specific site only. Low Methoxy Pectin (LMP) contains all those characters [11-14], so it was selected as a polymer in this work.

The main objective of this present study was to develop a multiple-unit, floating pulsatile drug delivery system for obtaining least drug release during floating in stomach and pulsed drug release in small intestine to achieve chronotherapeutic release of indomethacin for treatment of rheumatoid arthritis. To achieve this object, present work was carried out to formulate, characterize and evaluate calcium pectinate beads containing indomethacin by using various concentrations of natural polysaccharide of LMP with buoyancy imparting agent of sodium bi carbonate.

MATERIALS AND METHODS:

Materials:

Indomethacin was purchased from Yarrow Chemicals, Mumbai and Low methoxy pectin (LMP) was obtained as gift sample from Krishna Pectins Pvt. Ltd, Jalgoan. The other materials and solvents used in the present investigation were AR/LR grade.

Methods:

Formulation of indomethacin beads[11]

The hollow porous beads were prepared by dissolving the specified amount of LMP and specified amount of indomethacin in 10 ml of deionised water and specified amount of sodium bi carbonate was uniformly mixed as per table 1. The dispersion was sonicated for 30 min to remove any air bubbles. The resultant dispersion was dropped via a 23-gauge syringe needle (0.65 mm internal diameter) into 100 ml of 2% w/v calcium chloride solution containing 10% acetic acid. The content was stirred at 100 rpm using a magnetic stirrer for 15 min. The beads were filtered and washed three times with distilled water and subsequently oven dried at 50°C for 4 h. Beads of indomethacin were prepared using ionotropic gelation technique. This process was achieved by cross linking of indomethacin-low methoxy pectin dispersion with calcium ions to induce the spontaneous formation of calcium pectinate containing indomethacin beads. The prepared beads were collected and weighed.

Table 1: Formulation code for calcium pectinate beads of Indomethacin

MATERIALS	F_1	F_2	F_3
Drug(mg)	100	100	100
Low Methoxy Pectin(mg)	300	400	500
$NaHCO_3$: Polymer	0.5:1	0.5:1	0.5:1
$CaCl_2$(%)	2	2	2
Acetic acid(ml)	8	8	8

The percentage yield was calculated by the following formula,

Percentage yield = Actual weight of beads obtained / Total weight of excipients and drug X 100

Characterization of prepared beads Size analysis

The particle size of prepared beads was measured with an optical microscope fitted with a calibrated eyepiece. The mean of 100 beads was noted as particle size.

Bulk density and Tapped density

The bulk density and tapped density were determined with the help of a measuring cylinder by using the following formulas.

Bulk density = Weight of beads taken/ Bulk volume

Tapped density = Weight of beads taken/ Tapped volume

Drug content and Encapsulation efficiency [15, 16]

Twenty-five milligram beads of each batch was placed in 25 ml phosphate buffer, pH 7.4, and mechanically agitated on a shaker at 200 rpm for 3 h at 25^0C. The resultant dispersion was filtered through a Whatt's man filter paper 41 and analyzed at 318 nm using UV spectrophotometer. The encapsulation efficiency was determined by the following formula:

Encapsulation efficiency (%) = AQ/ TQ *100

Where, AQ is the actual drug content of the beads and TQ is the theoretical quantity of drug present in the beads.

Scanning Electron Microscopy

Morphological analysis or surface topography of beads was carried out using Scanning Electron Microscope (Field Instruments, Japan). Beads and their cross sections were coated with a thin gold palladium layer by a sputter-coater unit operated at an acceleration voltage of 10 kV

In-vitro Buoyancy study[17]

The *in-vitro* buoyancy study was characterized by floating lag time and total floating time. The time required for beads to raise to surface of dissolution medium and duration of time the beads constantly float on dissolution medium were noted. The test was performed by using a USP XXIII type II dissolution test apparatus. One hundred beads of each batch were placed in 900 ml 0.1 N hydrochloric acid (pH 1.2) containing 0.02% w/v tween 80. The media was maintained at 37°C ± 0.5 and stirred at 100 rpm. At hourly intervals, stirring was stopped for 2 minutes and the number of settled beads was counted visually.

Here, $t_{80\%}$ indicated time for which 80% of the beads remained floating.

***In-vitro* dissolution studies**[18, 19]

The dissolution studies of the indomethacin beads were performed using a USP XXIII type II dissolution test apparatus. The drug release study was carried out in 2 different media, 0.1 N HCl for time period equivalent to floating time ($t_{80\%}$) which varied for each batch and then followed by phosphate buffer pH 7.4 till the complete release of drug. Each 900 ml maintained at 37°C ± 0.5 and agitated at 100 rpm. Periodically, samples of 5 ml were withdrawn and were replaced with the same amount of fresh buffer to maintain sink conditions. The samples withdrawn were filtered through a Whatt's man filter paper 41 and measured spectrophotometrically at 318 nm.

Differential Scanning Calorimetrical (DSC) Analysis

A DSC study of pure drug indomethacin and drug loaded beads was carried out to detect possible polymorphic transition during the crystallization process. DSC measurements were performed on a DSC Q200, TA Instruments, USA. The analysis was performed at heating rate of 10 degree Celsius/min using nitrogen at flow rate of 50ml/min.

RESULTS AND DISCUSSION:

Formulation of floating pulsatile calcium pectinate beads

Three formulations (F1, F2 and F3) of indomethacin floating pulsatile calcium pectinate beads were formulated by simple ionotropic gelation technique. The beads were formed due to the cross-linking of the pectin with divalent calcium ions of the calcium chloride solution. The reaction between sodium bi carbonate and acetic acid occurred, liberating carbon di oxide as gas bubbles, which was responsible for floating of the beads and provide lag time release[19]

LMP has more capability to form insoluble rigid gel with divalent cation such as calcium ions cross-linked with galacturonic acid chain. This heterogeneous anionic polysaccharide has an ability to produce water-insoluble complex with drug. In stomach, pectin is not digested by gastric enzymes and has minimum swelling. But formulated calcium pectinate undergoes rapid swelling and gel relaxation at alkaline pH which is used in oral novel drug delivery system of site and time specific pulsatile release of indomethacin[11-14]. The percentage yield of the formulation was increased with increasing concentration of polymer (for F1, F2 and F3 were 78.2, 82.8 and 83.9 respectively.

Characterization of prepared beads

Particle size and morphological analysis

The particle sizes of beads were analyzed by optical microscopy and the mean particle sizes of beads were 1.546mm, 1.621mm and 1.772mm for F1, F2 and F3 formulations respectively. As the concentration of polymer increases during the preparation of beads the mean bead diameter also increases due to increase in relative viscosity at higher concentration of LMP and results formation of large droplets.

Drug loaded beads morphology was studied under Scanning Electron Microscopy (SEM). The dried beads were small and dense with a wrinkled circumference due to gradual water loss. The surface of beads was very rough and porous (Fig. 1-a). The cross section of beads showed a hollow core (Fig. 1-b). The thick boundaries in the beads may be due to the coalescence of the gas bubbles that pushed the internal matrix toward the periphery forming thick boundaries.

SEM images of optimized formulation

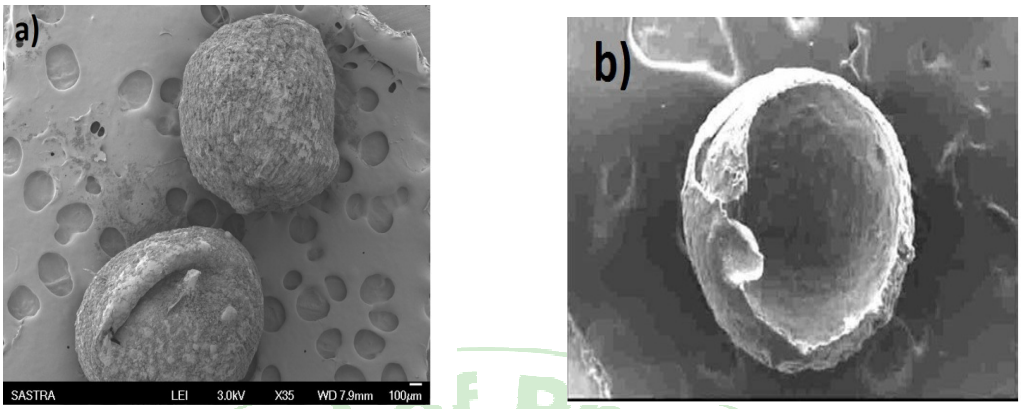

Figure 1: SEM image of Optimized formulation (a) and it's cross section (b)

Bulk Density and Tapped Density

Bulk densities of the beads are shown in table 2.

Table 2: Densities of prepared beads

Formulation Code	Bulk density (gm/ml)	Tapped density (gm/ml)
F1	**0.661**	**0.719**
F2	**0.657**	**0.721**
F3	**0.650**	**0.725**

The bulk density of the all beads was less than 1, because of inside gas entrapped hollow structure of beads. This may attributed to decrease in bulk density and made the beads to float on stomach pH. The bulk and tapped density of the beads showed good acceptable range indicating good packability. The density of the beads was decreased as the concentration of the polymer was increased which indicates that the beads formed at high polymer concentration are more compact and more porous than those prepared at low polymer concentration.

Drug content and Encapsulation efficiency

Drug content and Encapsulation efficiency of the formulations ranged from 140.25 mg to 155.14 mg and 70.13 to 77.5.5% respectively which was shown in table 3.

Table 3: Drug content and encapsulation efficiency

Formulation Code	Percentage yield (%)	Practical drug content(mg)	Theoretical Drug content(mg)	Encapsulation Efficiency (%)
F1	78.2	140.25	200	70.13
F2	82.8	145.37	200	72.65
F3	83.9	155.14	200	77.5

Nearly all the formulations has shown maximum of seventy percentage encapsulation efficiency. With an increase in polymer ratio, the increase in drug content and encapsulation efficiency were observed. This may be due to incorporation of sodium bi

carbonate in beads formulation along with increased polymer ratio, which evolutes carbon dioxide when in contact with acidic environment leads to the formation of hollow structure and during this entrapment leads to the coalescence of gas bubbles, which pushed the internal matrix towards periphery forming thick boundaries, minimizing drug leaching.

Buoyancy test

Beads of all batches has shown floating property without buoyancy lag time due to the presence of sodium bi carbonate in all the formulations. It was observed that with an increase in polymer concentration the floating time of the beads also increased (as 4, 5 and 6 hours for F1, F2 and F3 formulations respectively). This may be due to increased encapsulation of carbon dioxide within the polymer matrix making bursting of beads difficult for the liberation of carbon dioxide, making the beads floating for longer time in gastric pH. Floating time was primarily controlled by low density and porous nature of beads, which on turn is affected by both quantity of sodium bi carbonate and concentration of polymer solution. Here sodium bi carbonate concentration kept constant in all formulations. So there affected by the varying concentration of polymer solution the floating time ($t_{80\%}$) increases. This may be increases the concentration of polymer it may be decreases the bulk density and by increases the floating time of beads

***In- vitro* dissolution studies**

In-vitro dissolution study of all the formulations of indomethacin beads was carried out in two different media, first in pH 1.2 acidic buffer for time equivalent to floating time($t_{80\%}$) and then pH 7.4 phosphate buffer till to complete release. Finally data's were plotted represented in figure 2. Comparatively lesser drug release (3.69 – 4.58%) was observed at acidic pH. Because at acidic pH calcium pectinate remains protonated into insoluble form and indomethacin also insoluble in acidic media. But on next the complete drug release from beads of all batches occurred within one hour in phosphate buffer pH 7.4. Batches F1, F2, F3 showed 93.51%, 90.42%, 85.74% drug release at end of 60 minutes in phosphate buffer, as shown in figure 3. This second phase of the pulsed release in pH7.4 can be attributed to rapid swelling and gel relaxation of calcium pectinate at alkaline pH. Secondly, at above pH 7 indomethacin is freely soluble, which resulted in rapid and complete drug release could be useful in *in-vivo* absorption from large surface of small intestine.

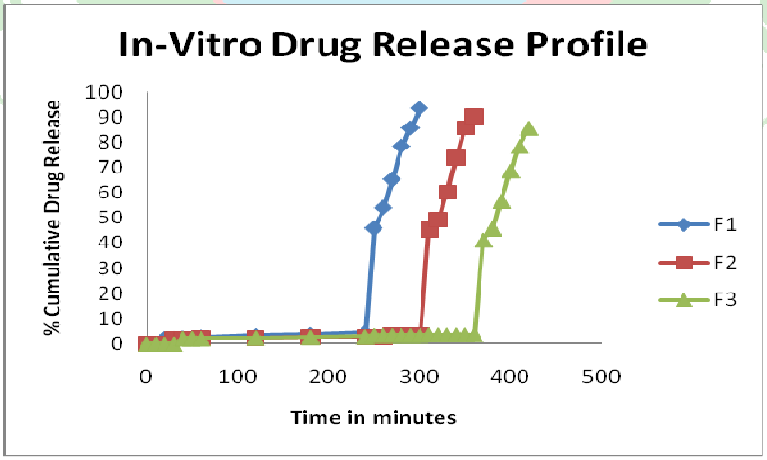

Figure 2: Comparative %cumulative drug release of formulations (F1-F3)

Differential Scanning Calorimetry (DSC)

DSC thermograms of indomethacin and loaded beads were presented in figure 3 & 4. Indomethacin showed sharp melting endotherm at 161.12^{0}C and drug loaded beads showed melting endotherm at 158.55^{0}C. This characteristic peak of indomethacin was within the limit (158-161.5^{0}C) and was not altered after

encapsulation indicating no chemical interaction between drug and polymer [20].

DSC thermogram of indomethacin

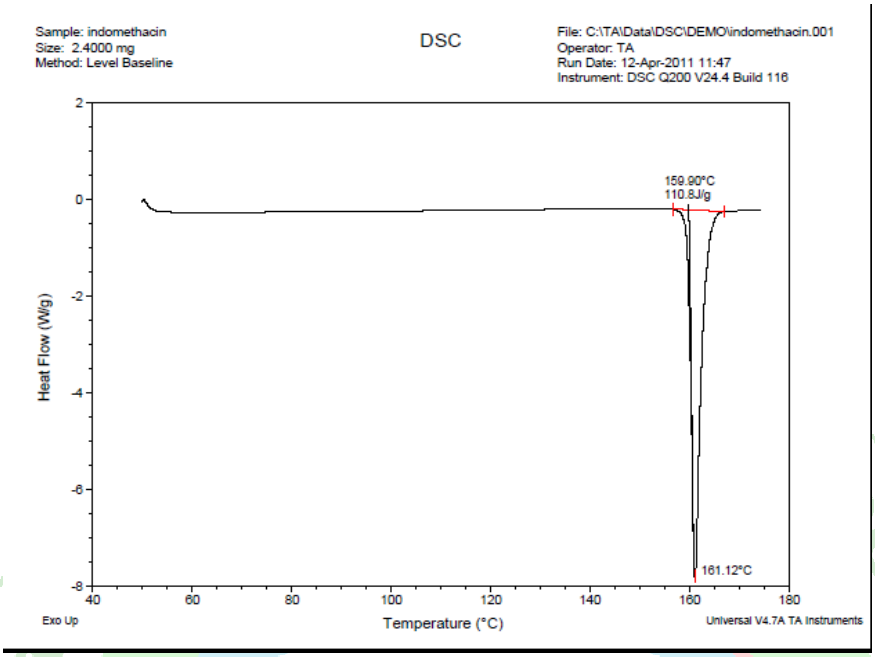

Figure 3: D.S.C. image of Indomethacin showed a characteristic endothermic peak at 161.12^{0}C, which corresponds to its melting point.

DSC thermogram of optimized formulation

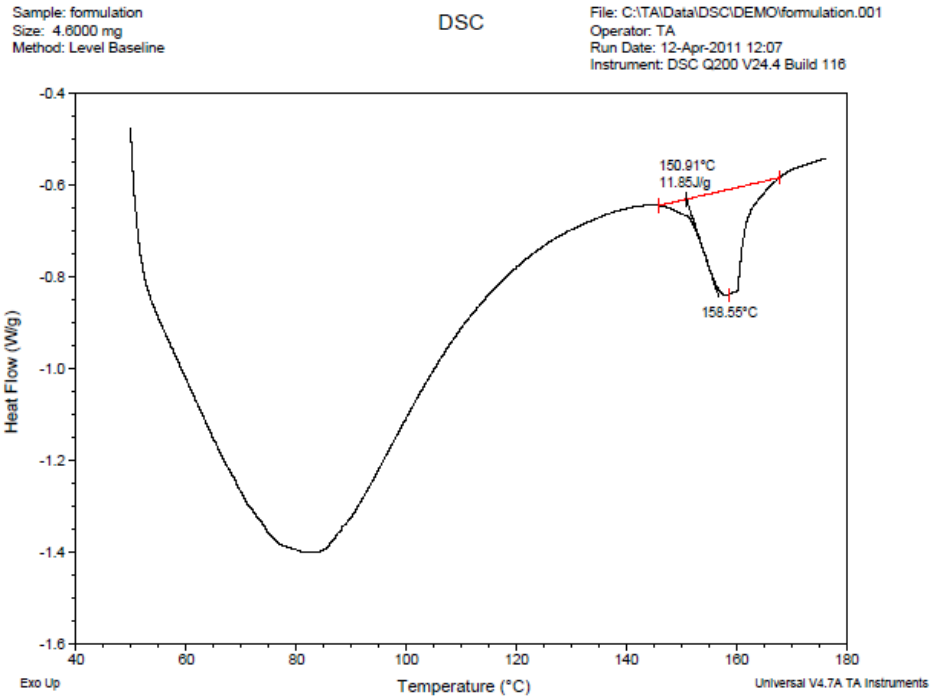

Figure 4: D.S.C. image of Optimized Formulation (indomethacin +LMP)

CONCLUSION

The site and time specific delivery of indomethacin beads have designed greater absorption in morning even though which administered at night which designed to release only in the early morning during the peak symptom of RA at specific absorption site of small intestine. Hence, it was concluded that this approach suggested the use of calcium pectinate beads of indomethacin for oral pulsatile drug delivery as the one of the promising site and time specific formulation technique in chronotherapeutic management of Rheumatoid Arthritis.

REFERENCES

1. *Kikuchi A, Okano T. Pulsatile drug release control using hydrogels. Adv Drug Del Rev 2002; 54: 53-77.*
2. *Lemmer B. Circadian rhythms and drug delivery. J Control Release 1991; 10: 63-74.*
3. *Herold M, Gunther R. Circadian rhythm of C-reactive protein in patients with rheumatoid arthritis. Prog Clin Biol Res 1987; 227: 271-279.*
4. *Rupali Singh, pramod Kumar Sharma, Rishabha Malviya. Circadian rhythms in Arthritis: A Review J Chr D D 2010; 1(1);19-25.*
5. *Brahmankar DM Jaiswal SB. Biopharmaceutics & Pharmacokinetics, 1st edition, New Delhi: Vallabh Prakashan; 1995: 347-348.*
6. *Davis SS, Prescott LF, Nimmo Ws, assessment of gastro intestinal transit and drug absorption. Novel drug delivery and therapeutic application. Wiley Colchester. 1989; 89-101.*
7. *Meyer JH, Dress man J, Fink AS, Amidon G. Effect of size and density on gastric emptying of indigestible solids. Gastro enterology 1985; 89: 805-813.*
8. *Rodrignaz M, Vila Jato JL, Torres D. Design of a new multiparticulate system for potential site specific & Controlled Drug Delivery to the Colonic region. J Cont Release 1998; 55: 67-77.*
9. *Kumar R. Kurumaddali, Guru V. Betageri and William R. Ravis. Preparation and dissolution characteristics of indomethacin sustained release beads. Drug Development and Industrial Pharmacy 1992 ; 18(15): 1709-1717.*
10. *Ibekwe VC, Fadda HM, MC Connell EL, Khela MK, Evans DF, Basit AW. Interplay between pH responsive ileo-colonic release sysem. Pharm Res 2008; 25 (8): 1828-35.*
11. *Sharddha S.Badve, Praveen Sher, Aruna Korde, Atmaran P. Pawar. Development of hollow/ porous calcium pectinate beads for floating-pulsatile drug delivery. Eur J Pharm & BioPharm 2007; 65: 85-93.*
12. *Srimornsak P, Prakongpam S, Puttipatkhachorm S. Calcium pectinate gel coated pellets as an alternative carrier to calcium pectinate beads. Int J Pharm 1997; 156: 189-194.*
13. *Sriamornsak, P, Nanthanid J. Calcium pectinate gel beads for controlled release drug delivery: I. Preparation & invitro release studies. Int J Pharm 1998; 160: 207-212.*
14. *Wong T W, Lec H Y, Chan L M, Heng P W S. Drug release properties of pectinate microspheres prepared by emulsification method. Int J Pharm 2002; 242: 233-237.*
15. *Akhgari A, Afrsiabi Garekani H, Sadeghi F, Azimaie M. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Inter J Pharm 2005; 305: 22-30.*
16. *Praveen Sher, Ganesh Ingavle, Surendra Ponrathnam and Atmaram P. Pawar. Low density porous carrier based conceptual drug delivery system. Microporous and Mesoporous Materials 2007; 102 (1-3): 290-298.*
17. *Priya Bhat, Dipti patel, Rakesh chaudhari. Formulation and Evaluation of floating beads for chronotropic delivery of lornoxicam. World J Pharm Res 2012; 1(3): 738-756.*
18. *Sameer Sharma, Atmaram Pawar. Low density release of pulsatile system for multiparticulate meloxicam. Int J of Pharm 2006; 313: 150-158.*
19. *Anand Panchakshari Gadad, Annapureddy Dasaratharami Reddy, Panchaxari Mallappa Dandagi, Vinayak S Masthiholimath. Design and characterization of hollow/porous floating beads of captopril for pulsatile drug delivery. Asian J Pharm 2012; 137-143.*
20. *Analytical Profiles of Drug Substances. Volume 13, edited by Klaus Florey, Academic Press, London: 2005; 211- 229.*