CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

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

FORMULATION AND EVALUATION OF DUAL CROSS-LINKED PULSATILE BEADS FOR CHRONOTHERAPY OF RHEUMATOIDARTHRITIS

[1]Namrata Gupta, [2]Sanjay kumar Dhaker

[1]NRI Institute of research and technology-pharmacy

Article Received: October 2021 **Accepted:** November 2021 **Published:** December 2021

Abstract:

The present study was aimed at exploring the feasibility of time and pH dependent dual cross- linked pulsatile beads based drug delivery system of Lornoxicam to modulate the drug level in synchrony with the circadian rhythm of arthritis. In the present research work, the researcher had at tempted to develop novel dosage forms by using chrono pharmaceutical approach. A dual cross- linked pulsatile dosage form with 7-8 hrs lag time, taken at bedtime and start of drug release in early morning hours, can prevent the early morning stiffness and the sharp increase of pain in arthritic patients during the early morning hours and further maintenance for a longer period of time, thereby reducing the frequency of administration and hence assure patient compliance. The Pre formulation studies like pH, melting point, solubility UV-analysis and FTIR study of Lornoxicam were found to comply with of facial standards. The FTIR Spectra revealed that there was no interaction between the polymers and drug. Polymers used were compatible with Lornoxicam. Surface smoothness of the Lornoxicam beads was enhanced by increase in the polymer concentration, which was confirmed by SEM. Increase in amount of polymer increased the particle size and drug entrapment efficiency of the Lornoxicam beads. Invitro drug release of beads showed biphasic release pattern with initial minimum burst release effect, which may be attributed to the Lornoxicam loaded on to surface of the particles.

Keywords: *Pulsatile release, solubility, partition coefficient, melting point, Bioavailability.*

Corresponding author:

Namrata Gupta

NRI Institute of research and technology-pharmacy.

QR code

Please cite this article in press Namrata Gupta et al, ***Formulation And Evaluation Of Dual Cross-Linked Pulsatile Beads For Chronotherapy Of Rheumatoid Arthritis.,*** *Indo Am. J. P. Sci, 2021; 08(12).*

INTRODUCTION:

Controlled drug delivery systems have acquired a centre stage in the arena of pharmaceutical research and development sector. Such systems offer temporal an Controlled drug delivery systems have acquired a centre stage in the arena of pharmaceutical research and development sector. Such systems offer temporal and/or spatial control over the release of drug and grant a new lease of life to a drug molecule in terms of patentability. Oral controlled drug delivery systems represent the most popular form of controlled drug delivery systems for obvious advantages of oral route of drug administration. These dosage forms offer many advantages, such as nearly constant drug level at the site of action, prevention of peak-valley fluctuation, reduction in dose of drug, reduced dosage frequency, avoidance of side effects and improved patient compliance. In such systems the drug release commences as soon as the dosage form is administered as in the case of conventional dosage forms. However, there are certain conditions, which demand release of drug after a lag time. Such a release pattern is known as "pulsatile release" (Gothaskar et al., 2004).

MATERIALS AND METHODS:

Materials:

The following materials of Pharma grade or the best possible Laboratory Reagent (LR) were used as supplied by the manufacturer. The doubled is tilled water was used in all experiments.

Table 1: List of instruments used

S. No.	Instrument	Manufacturer
1.	U.V Visible spectrophotometer	Systronic, India
2.	FTIR spectrophotometer	Shimadzu Corporation, Japan
3.	Magnetic stirrer	Remi motors, Ahmedabad
4.	Mechanical stirrer	Remi motors, Ahmedabad
5.	Centrifuge	Remi motors, Ahmedabad
6.	SEM, JSM – 840A	JEOL, Japan
7.	Electronic balance	Citizen scales Pvt. Ltd
8.	Digital pH meter	Digisun Electronics, Hyderabad
9.	Digital melting point apparatus	Jyoti Scientific , India
10.	USP dissolution apparatus	Electrolab TDL-08L
11.	Hot air oven	Techno scientific, Bangalore
12.	Microscope	Jyoti Scientific , India
13	U.V Visible spectrophotometer	Systronic, India
14	FTIR spectrophotometer	Shimadzu Corporation, Japan

Table 2: List of chemicals used with grade and supplier

S. No.	Materials used	Grade	Manufacturer
1.	Lornoxicam	Pharma Grade	Hetro lab limited(Hyderabad, India)
2.	Pactin	Pharma Grade	SD fine chemicals,Mumbai
3.	Sodium Alginate	Pharma Grade	SD fine chemicals,Mumbai
4.	EudragitL-100	LR	Shreeji chemicals, Mumbai
5.	EudragitS-100	LR	Shreeji chemicals, Mumbai
6.	Methanol	LR	SD fine chemicals,Mumbai
7.	Ethanol	LR	SD fine chemicals,Mumbai
8.	Hexane	LR	SD fine chemicals,Mumbai
9.	Acetone	LR	SD fine chemicals,Mumbai
10.	Petroleum ether	LR	SD fine chemicals,Mumbai
11.	Sodium hydroxide Pellets	LR	Qualigens fine chemicals, Bombay
12.	Hydrochloric acid	LR	SD fine chemicals,Mumbai
13.	Potassium dihydrogen phosphate	LR	Qualigens fine chemicals, Bombay
14.	Potassium chloride	LR	Qualigens fine chemicals, Bombay

Methods:

Preformulation studies:

Preformulation testing is the first step in the rationale development of dosage forms of a drug substance. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulator in developing stable and bioavailable dosage forms, which can be mass- produced.

Description of Lornoxicam:

It is the initial evaluation during preformulationstudies which assess the colour, odor and taste of the substance. This was only a descriptive test.

Determination of pH:

The pH of Lornoxicam was determined using digital pH meter for freshly prepared 1% solution of Lornoxicam in Di chloro methane : Methanol (4:1).

Determination of melting point:

Melting point of the drug was determined by taking small amount of drug inacapillary tube closed at one end. The capillary tube was placed in a melting pointapparatus and the temperatureat which drug melts was recorded. This was performedthrice and average value was noted.

Determination of solubility:

Solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of drug was determined in various common solvent.

Determination of partition coefficient:

25 mg of drug and n-octanol (25 ml) and phosphate (25 ml) buffer (pH 7.4) were taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug was calculated.

Drug polymer interaction (FTIR) study:

FTIR spectroscopy was performed on Fourier trans for min frared spectrophotometer (Shimadzu, Japan). The pellets of powdered formulation and potassium bromide were prepared by compressing the powders at 20 psi for10 min on KBr-press and the spectra were scanned in the wave number range of 4000-600cm^{-1} FTIR study was carried out on

Lornoxicam, polymers and physical mixture of Lornoxicam and polymers.

Determination of λ_{max} of Lornoxicam:
Standard stock solution of Lornoxicam was prepared by dissolving accurately weighed100mg of Lornoxicam in the little equantity of 0.1NH Clin 100mlvolume tric flask. The volume was then made up to mark by using 0.1NHCl, so as toget the solution of 1000µg/ml. From the standard stock solution, 2ml was diluted to100ml with 0.1NHCl. There sulting solution containing 20µg/ml was scanned between 200 to 400nm and λ max of Lornoxicamin 0.1NHCl was observed.

Standard calibration curves for Lornoxicam:
Standard stock solution of Lornoxicam was prepared by dissolving accurately weighed 100mg of Lornoxicam in the little equantity of 0.1NHCl in 100ml volumetric flask. The volume was then made up to mark by using 0.1NHCl, so as to get the solution of 1000µg/ml. From the standard stock solution. From the stock solution serial dilutions were do neto obtain solutions in the conc ranging from 5 to 25µg/ml. The absorbance of the solution was measured at 376 nm using UV-visible spectrophotometer. A graph of concentration v/s absorbance was plotted. Similarly, standard calibration curve of Lornoxicam were also prepared in pH 6.8 buffers by using the above said method.

Formulation and Design of Dual Cross-Linked Bead:

Ionotropic gelation method was used for the preparation of Lornoxicam loaded beads (Bansal and Pande, 2013). Lornoxicam was dispersed in distilled water and sonicated for 45 sec for reducing the particle side of drug (Table 5.1). Preweighted amount of pectin and sodium alginate were completely dissolved into the above solution and stirred for few minutes. The uniform dispersion was carried out by dispersion dropping with 25 gauge needles into 5% calcium chloride solution and stirred for 10 min for crosslinking of pectin and alginate. After the required cross-linking time, beads were filtered, washed with 50 mL double distilled water, and dried in the incubator at C till the weight is constant.

Prepared Ca^{2+} single cross-linked pectin alginate beads (F6 and F7)were immediately transferred into a 50 mL solution of 5% aluminum chloride (% w/v) for 5 min and then the Ca^{2+} and Al^{3+} dual cross-linked beads were separated by filtration, washed thrice with 50 mL double distilled water, air dried, and finally vacuum-dried at 40°C till constant weight.

Enteric-coated gelatin capsules were prepared by manual dipping technique. Entire hard gelatin capsule was dipped in 2% w/v EudragitS100 & Eudragit L100 (1: 2) in methanol. The coated gelatin capsule was dried at 40°C for 30 min. Equivalent amount (8 mg) of dual cross-linked beads were filled along with 10% magnesium stearate as a lubricant in size 2 of enteric-coated capsule.

Table 3: Compositions of Beads Formulations

Formulation Variables	Formulation Batch Code							
	F1	F2	F3	F4	F5	F6	F7	F8
Lornoxicam (mg)	50	50	50	50	50	50	50	50
Pectin (mg)	100	150	200	50	250	100	100	100
Sodium alginate (mg)	400	350	300	450	250	400	400	400
Distilled water (ml)	10	10	10	10	10	10	10	10
$CaCl_2$ (% w/v)	5	5	5	5	5	5	5	5
$AlCl_3$ (% w/v)	-	-	-	-	-	5	5	5
Cross linkingtime (min.)	10	10	10	10	10	10	15	20

Evaluation of Lornoxicam Beads:

Particle size distribution:

The particle size determination of calcium alginate beads was carried out using an optical microscope along with a stage micrometer having an accuracy of 0.01 mm. A suspension of beads in liquid paraffin was prepared in a beaker and then one drop of this was dropped on a clean glass slide and covered with a cover slip. The average sizes of 100 beads were determined for each formulation using the calibration factor. The average diameter of the beads was calculated.

Morphological Study:

The surface morphology of drug-loaded beads by using a scanning electron microscope (model JEOL JSM-6360, Japan). The beads were mounted on an appropriate stub and then coated with carbon and gold (100 and 50 Å thickness respectively) sputter module in a vacuum evaporator in an argon atmosphere. The coated samples were then observed under a scanning electron microscope.

Drug Content

For determining drug content, weighed amount (35 mg) of dual cross- linked pectin-alginate beads were placed into the 100 mL of 0.1 N NaoHsolution of each formulation and stirred the solution with mechanical stirrer then filtered the solution and analyzed spectrophotometrically at 376 nm in UV spectrophotometer.

Percentage yield:

The production yield of prepared dual cross-linked pectin-alginate beads was calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of beads and percent yields were calculated as per the following formula:

% yield = (Actual weight of product / Total weight of excipient and drug) x 100

Percent Entrapment efficiency:

Known amount of dual cross-linked pectin-alginate beads were placed into the 100 mL of 0.1 N NaoH solution of each formulation and stirred the solution with mechanical stirrer then filtered the solution and analyzed spectrophotometrically at 377.5 nm in UV spectrophotometer and % Entrapment efficiency was calculated.

Swelling Study:

Swelling behavior of pectin alginate dual cross-linked beads was studied by using digital caliper. All formulated batches were placed in 1.2 pH buffer and 6.8 pH buffer in Petridis and the diameter of 10 beads was measured after and before the swelling of beads at predetermined time interval, and swelling (%) was estimated.

DSC Study:

DSC thermo grams of lornoxicam, physical mixture, and pectin alginate beads were recorded using differential scanning colorimeter (DSC-60, Shimadzu, Japan). Each sample (5–10 mg) was scanned in pierced Al pans. The measurement was performed between 50 and 400 ^{o}C at heating rate 10 ^{o}C/min.

In-vitro dissolution studies

USP dissolution apparatus I (Dissolution test TDT-08L plus, Electrolab, India) was used to perform the release of dual cross-linked beads. Basket was rotated at 50 rpm and temperature maintained at C. Dissolution studies were carried out in 900 mL of 0.1 N hydrochloric acid buffer, 1.2 pH for 2 hour and pH 6.8 phosphate buffer for remaining hours. After 2 hours 1.2 pH hydrochloric acid buffers was replaced with 6.8 pH phosphate buffer and study was carried out for 24 hours. Samples (5 mL) were withdrawn and filtered. The withdrawal sample was replaced with equal volume of fresh medium at regular time interval. The amount of drug release was analyzed spectrophotometrically at 376 nm.

RESULT AND DISCUSSION:

Preformulation Studies:

Description:

The colour, odour, nature and taste of the API were evaluated. It was found to be orange to yellow crystalline powder, as per the monograph.

pH determination:

The pH of Lornoxicam in water was obtained 3.8.

Melting point determination:

Melting point for Lornoxicam was found to be 228.5 ^{o}C. The official reported melting point range for is 225-230^{o}C. Hence, an experimental value nearly matches with reported values.

Solubility study:

Since lornoxicam is a weak acid (pKa of 4.7), the aqueous solubility of lornoxicam is pH dependent. Increasing pH leads to decrease in the ratio of non- ionized to ionized drug, and in solubility-pH profiles, the solubility of lornoxicam decreases exponentially with the increase of pH from alkaline pH 9.0 to acidic pH 3.0.The solubility of Lornoxicamin10mg/10ml of solvent was carried out and it revealed that it

is soluble in Dichloromethane:Methanol(4:1) and 0.1NNaOH.

Determination of partition coefficient:
The Partition Coefficient of lornoxicam was found 1.8 in n-octanol and phosphate buffer (pH 7.4).

Determination of λmax:
The λmax of Lornoxicam was found to be 376nm (Figure 1).

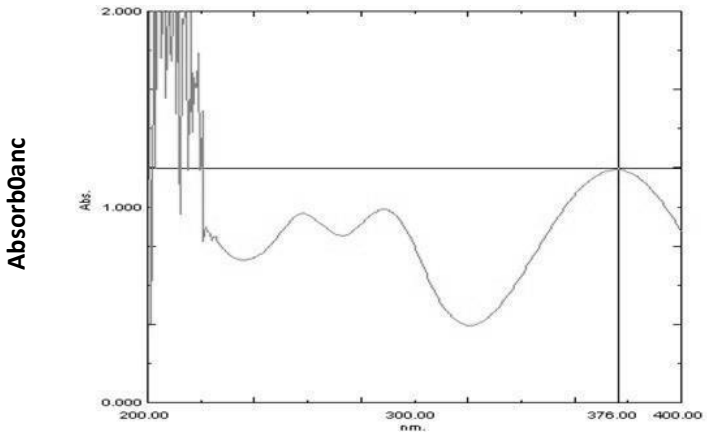

Figure 1: λmax of Lornoxicam

FTIR study of Lornoxicam:
The FTIR Spectrum of Lornoxicam was found to be similar to the standard spectrum of Lornoxicam.The spectrum of Lornoxicam shows the following functionalgroups at their frequencies.

Standard calibration curve of Lornoxicam:
A standard calibration curve for the Lornoxicam was obtained by measuring absorbance at 376 nm in media 0.1 N HCl, pH 1.2 and pH 6.8 buffer by plotting the graph of absorbance v/s concentration. The standard plots of Lornoxicam show good linearity.

Table 1: Standard calibration curve of Lornoxicamin 0.1 N HCl

S. No	Concentration (µg/ml)	Absorbance
1.	2	0.08
2.	4	0.17
3.	6	0.26
4.	8	0.34
5.	10	0.44

Figure 2: Standard calibration curve of Lornoxicam in 0.1M H

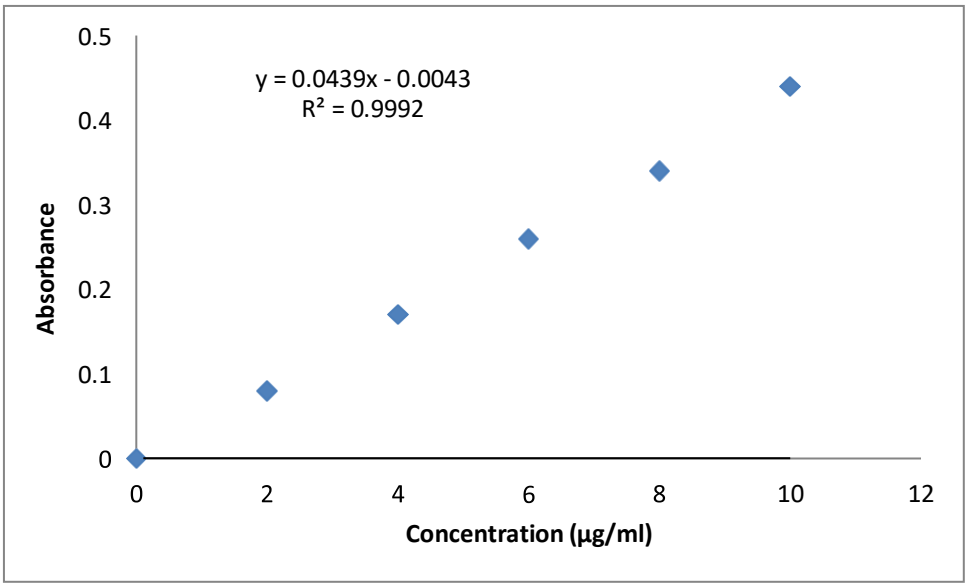

Table 2: Standard calibration of Lornoxicam in 6.8 pH buffer

S. No	Concentration (µg/ml)	Absorbance
1.	2	0.21
2.	4	0.32
3.	6	0.55
4.	8	0.69
5.	10	0.81

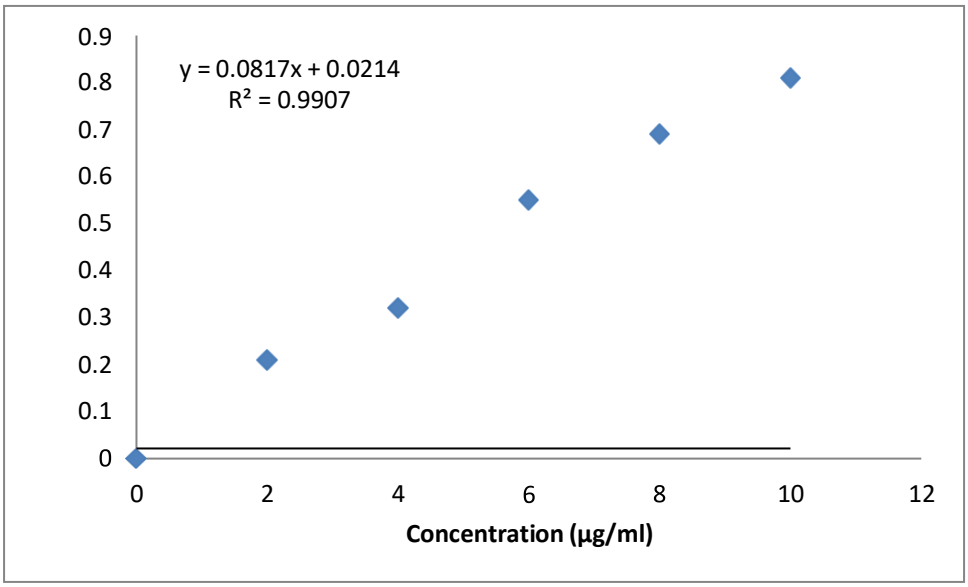

Figure 3: Standard calibration curve of Lornoxicam in 6.8 pH buffer

Formulation Development and Designing of Dual Cross-Linked Bead:

Morphological and Particle Size Study:

The surface morphology of the Lornoxicam beads was studied by SEM. Surface smoothness of the Lornoxicam beads was increased by increasing the polymer concentration, which was confirmed by SEM. All formulation showedsmooth and wrinkled free surface.

As the Lornoxicam to polymer ratio was increased, the mean particle size ofthe beads was also increased. The significant increase may be due to the increase inthe viscosity of the droplets (may be due to the increase in concentration of polymer solution). Photographic images were also obtained, showed good sphericity.

Table 3: Average diameter of Lornoxicam beads

S. No.	Formulation	Average Size (µm)
1.	F1	848.12
2.	F2	779.42
3.	F3	758.72
4.	F4	810.25
5.	F5	778.84
6.	F6	998.05
7.	F7	858.62
8.	F8	878.13

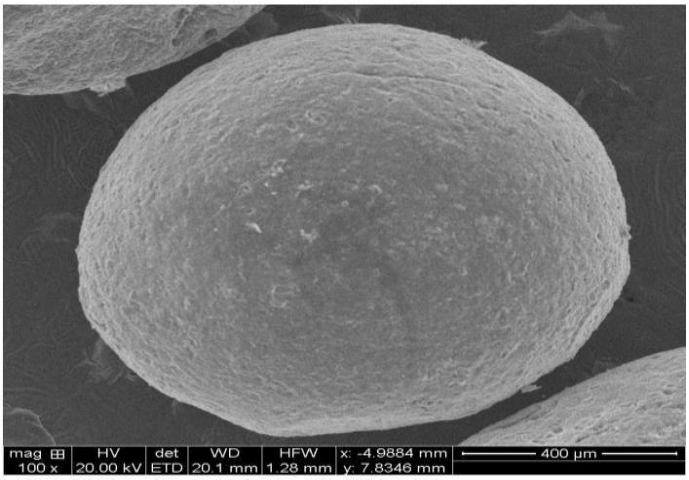

Figure 4: SEM Photographs of Lornoxicam Beads (F4)

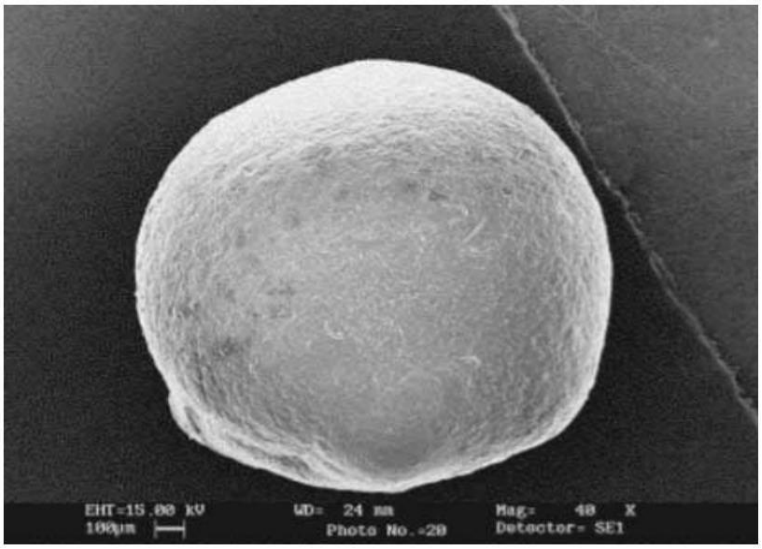

Figure 5: SEM Photographs of Lornoxicam Beads (F7)

Figure 6: Optical Photographs of Lornoxicam Beads (F4)

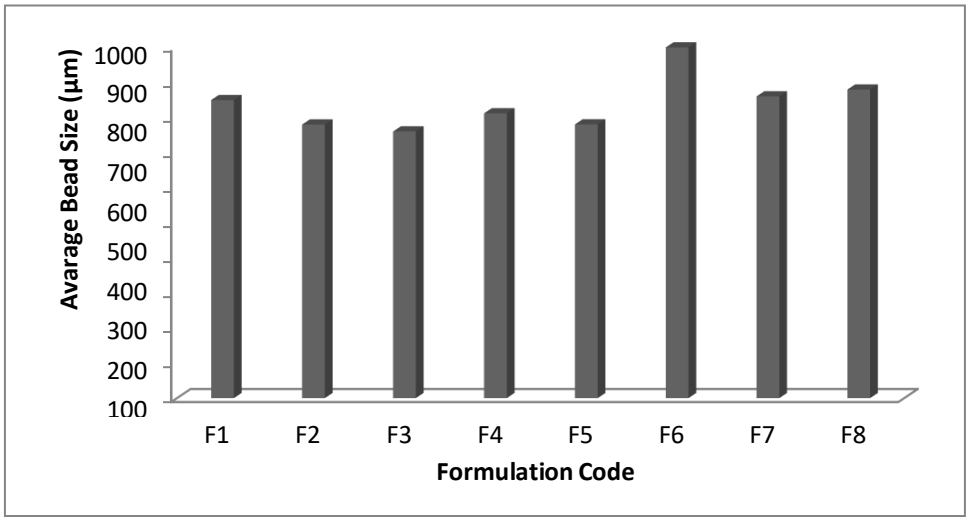

Figure 7: Average Diameter of Lornoxicam Beads

Drug Content, Percentage Yield and Entrapment Efficiency:
Drug content for Lornoxicam beads were 62.02, 52.34, 53.96, 58.44, 55.76, 62.35, 59.73 and 62.08 for formulation F1, F2, F3, F4, F5, F6, F7 and F8 respectively. Drug entrapment efficiency increased with increase in the polymer concentration. This might be due to the higher viscosity, which restrict drug to migrate during formulation processing. The percent of drug content in the formulations were found to be in the range of 53.98 % to 62.35% The percentage entrapment efficiency was found to be 80.99 to 72.87. The percent yields were found in between 83.28%to91.34% The results obtained are given in Table.6.9 and their histograms shown in Figure 6.10, 6.11 and 6.12 respectively. A maximum of drug entrapment efficiency was obtained in the Lornoxicam beads which were prepared by using, higher polymer concentration. It was further observed that the drug entrapment was proportional to the drug. polymer ratio and the size of the beads. By increasing the polymer concentration,the encapsulation efficiency was also increased.

Table 4: Drug entrapment efficiency of Lornoxicam beads

S. No.	Formulation	Drug Content	Percent Yield	Entrapment Efficiency
1.	F1	62.04	82.28	80.83
2.	F2	52.24	88.36	70.81
3.	F3	52.96	84.63	72.67
4.	F4	57.44	89.47	77.25
5.	F5	54.76	87.39	74.57
6.	F6	61.35	90.34	81.22
7.	F7	58.73	85.34	78.54
8.	F8	61.08	87.62	80.89

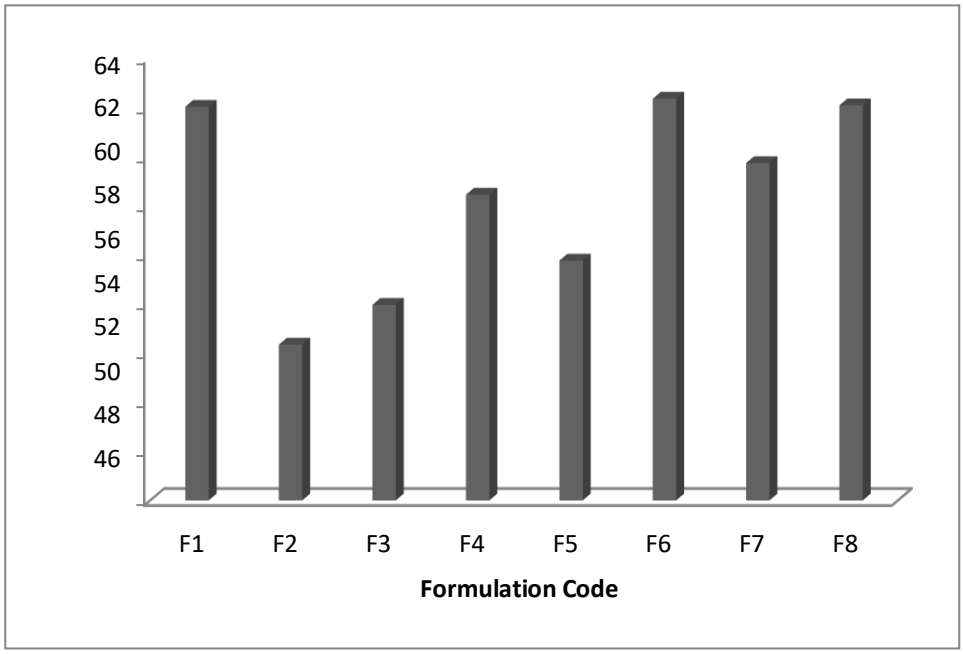

Figure 8: Drug Content of Lornoxicam Beads

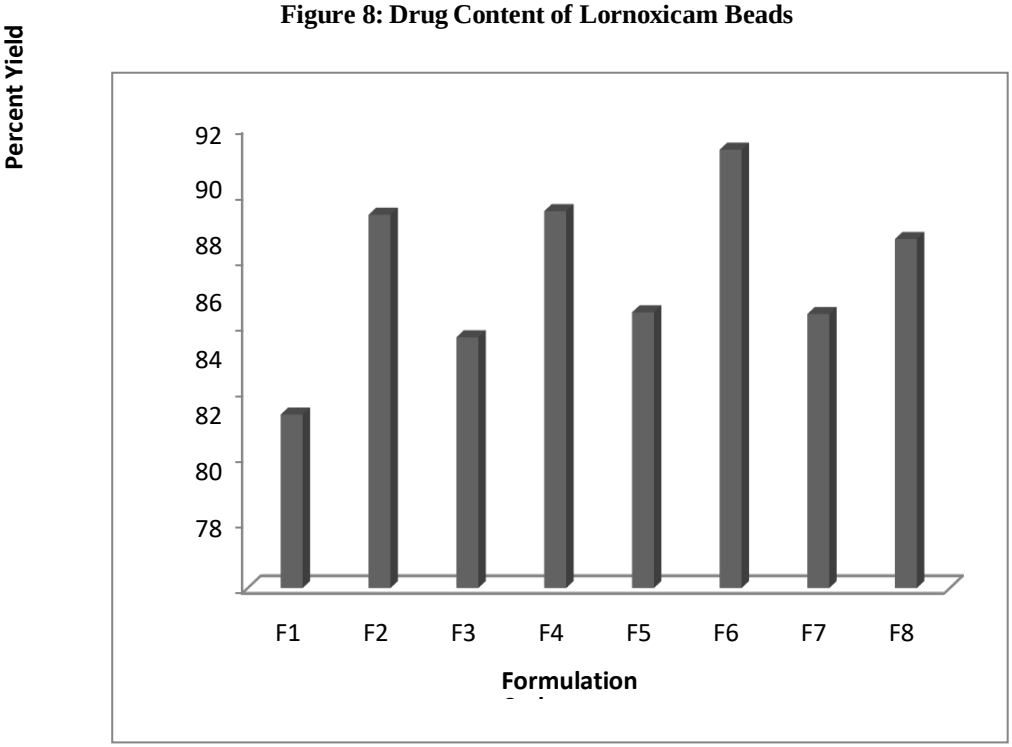

Figure9: Percent Yield of Lornoxicam Beads

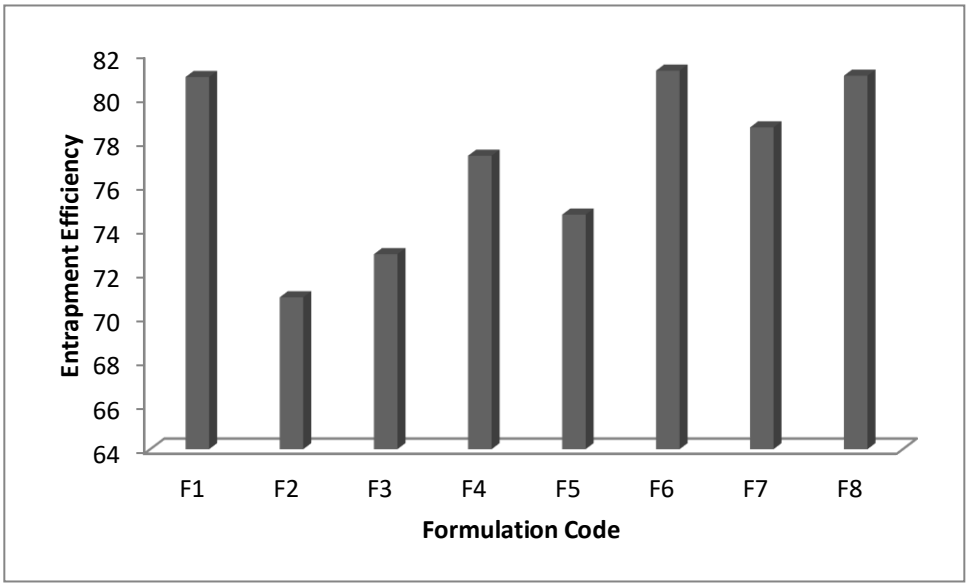

Figure 10: Drug Entrapment Efficiency of Lornoxicam Beads

Swelling Study:

Swelling property was mostly affected by the concentration of sodium alginate, pectin, and cross-linking of beads. As the concentration of sodium alginate increases the swelling capacity of beads increases considerably while the swelling capacity is rendered by cross-linking. Formulation F1, F4 and F6, F7 have same concentration of sodium alginate but F1 and F4 show higher swelling ability compared to F6 and F7 due to the cross-linking. It is observed that the dual cross- linking beads have lower degree of swelling ability than single cross-linked beads. Also, as the concentration of sodium alginate 450 mg in formulation F4 reduces to 250 mg in F5, the percentage swelling get reduced. Swelling ability of beads was observed lesser in pH 1.2 as compared to pH 6.8.

Table 5: Drug entrapment efficiency of Lornoxicam beads

S. No.	Formulation	Percent Swelling (1.2 pH)	Percent Swelling (6.8 pH)
1.	F1	30.22	197.19
2.	F2	39.51	172.11
3.	F3	41.16	155.67
4.	F4	23.05	290.04
5.	F5	54.11	111.72
6.	F6	28.23	53.61
7.	F7	24.44	95.71
8.	F8	27.51	78.87

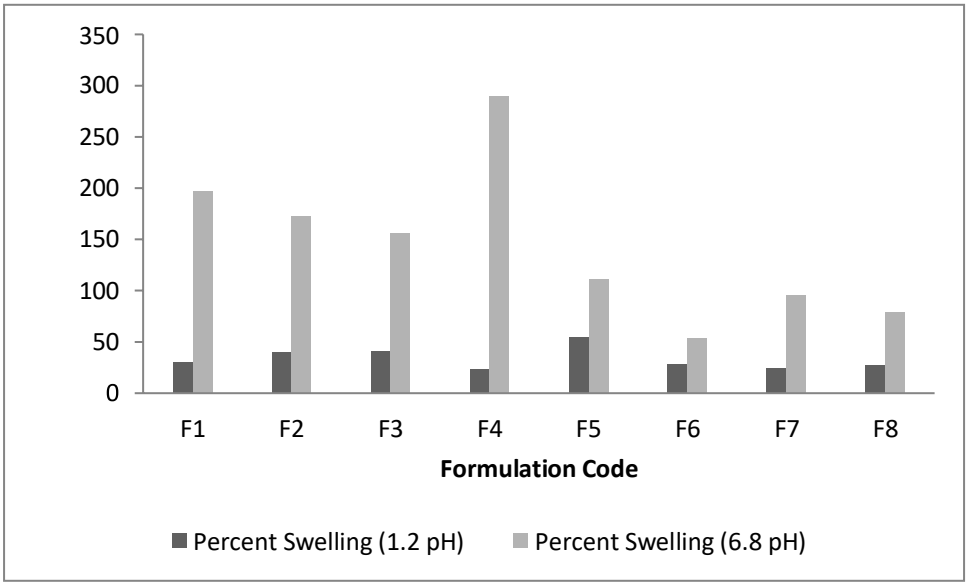

Figure 11: Swelling study of Lornoxicam Beads

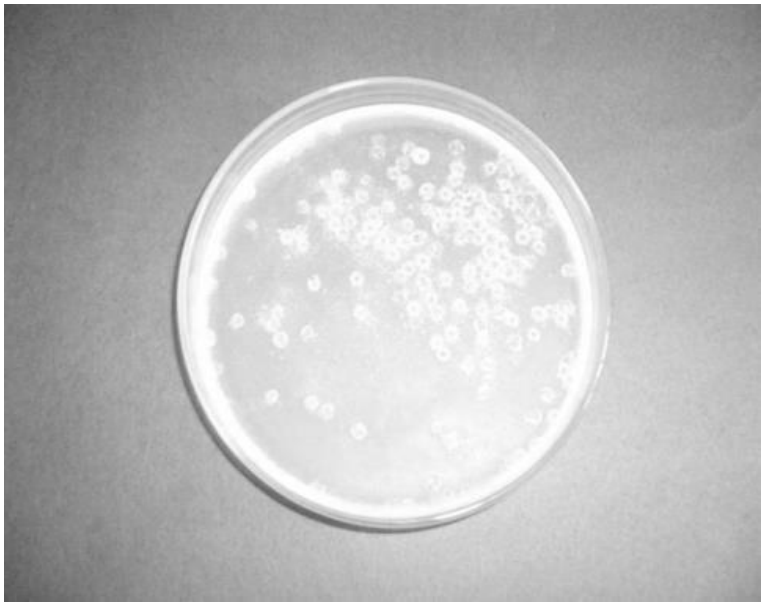

Figure 12: Digital Photographs of Swelling Study of Lornoxicam Beads inpH 1.2 after 2 hours.

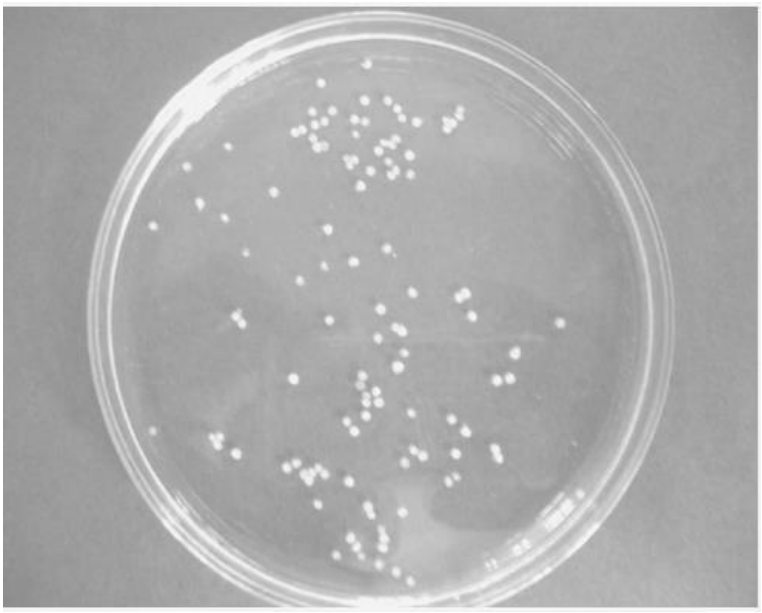

Figure 13: Digital Photographs of Swelling Study of Lornoxicam Beads in pH 6.8 after 8 hours.

Drug Polymer Interaction (FTIR)Study:

From the spectra of Lornoxicam and physical mixture of Lornoxicam and polymers, it was observed that all characteristic peaks of Lornoxicam were present inthe combination spectrum, thus indicating compatibility of Lornoxicam and polymer.

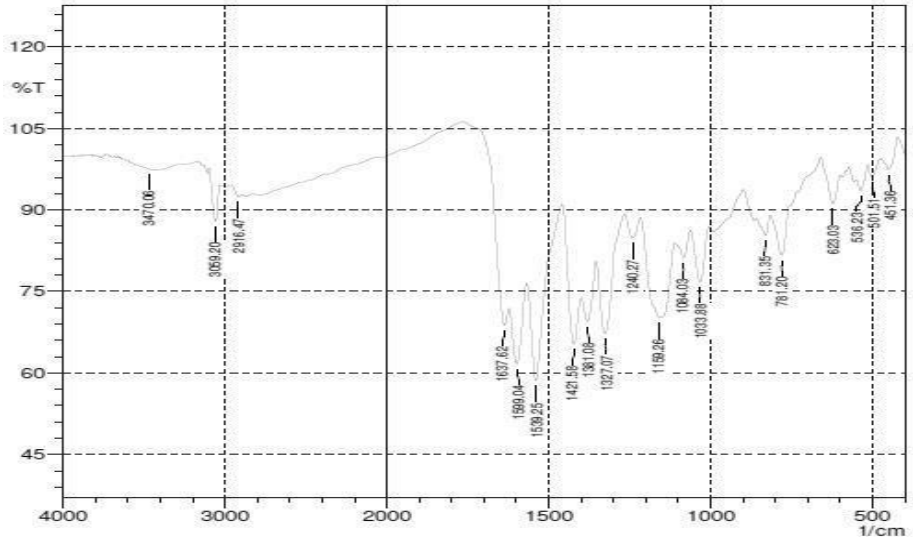

Figure 14: FTIR Spectrum of Lornoxicam

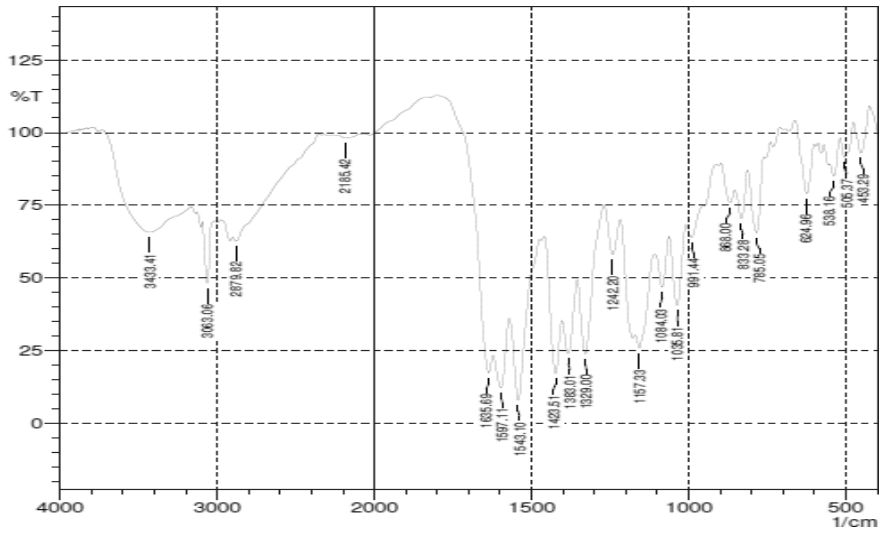

Figure 15: FTIR Spectrum of Physical Mixture of Lornoxicam, Pectin & Sodium Alginate.

Table 6: FTIR Spectrum Data of Lornoxicam and Physical Mixture of Lornoxicam and Polymers

S. No.	IR Spectrum	Peaks(cm-1)	Groups	Stretching/ Deformation
1.	Lornoxicam	3470.06	N-H	Stretching
		1637.62	C-O	Stretching
		1599.04	C=C	Stretching
		1327.07	C-N	Stretching
		1033.88	S=O	Stretching
		831.35	C-Cl	Stretching
2.	Physical mixture of Lornoxicam and Polymers	3433.41	N-H	Stretching
		1635.69	C-O	Stretching
		1597.11	C=C	Stretching
		1329.00	C-N	Stretching
		1035.81	S=O	Stretching
		833.28	C-Cl	Stretching

DSC Thermogram:

In order to confirm the physical state of Lornoxicam in the beads, DSC of Lornoxicam, and Lornoxicam beads

were carried out as shown in Figure 6.17 to 6.18. The DSC trace of Lornoxicam showed a sharp end othermic peak at 215.51°C,its melting point. The DSC thermograms of the polymers also showed peaks at their respective melting points. The absence of sharp end othermic peak of Lornoxicam at 215.51°C in the DSC of the Lornoxicam beads suggests that Lornoxicam existed in anamorphous or disordered crystalline phase as a moleculardispersion in polymeric matrix..

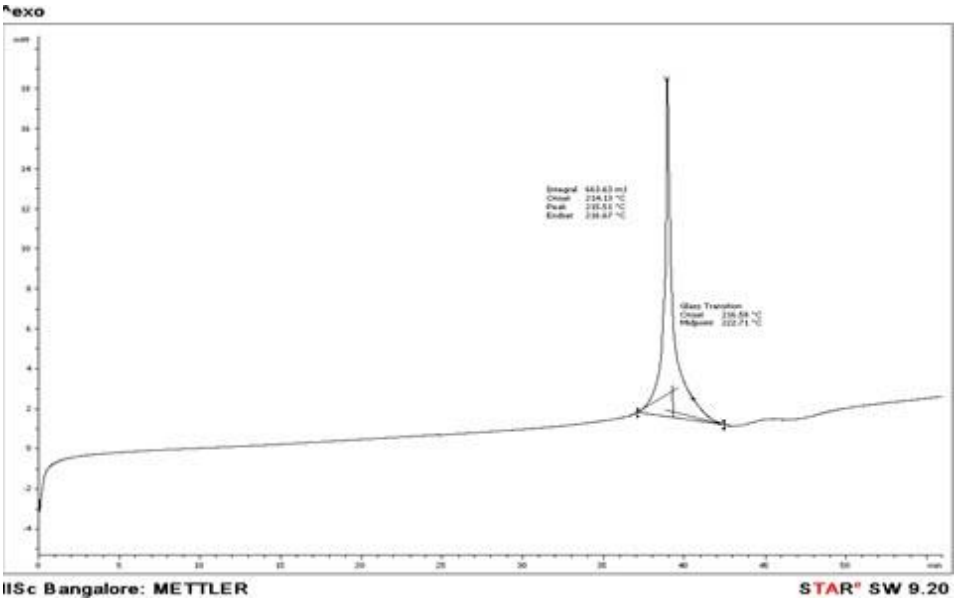

Figure 16: DSC Thermogram of Lornoxicam

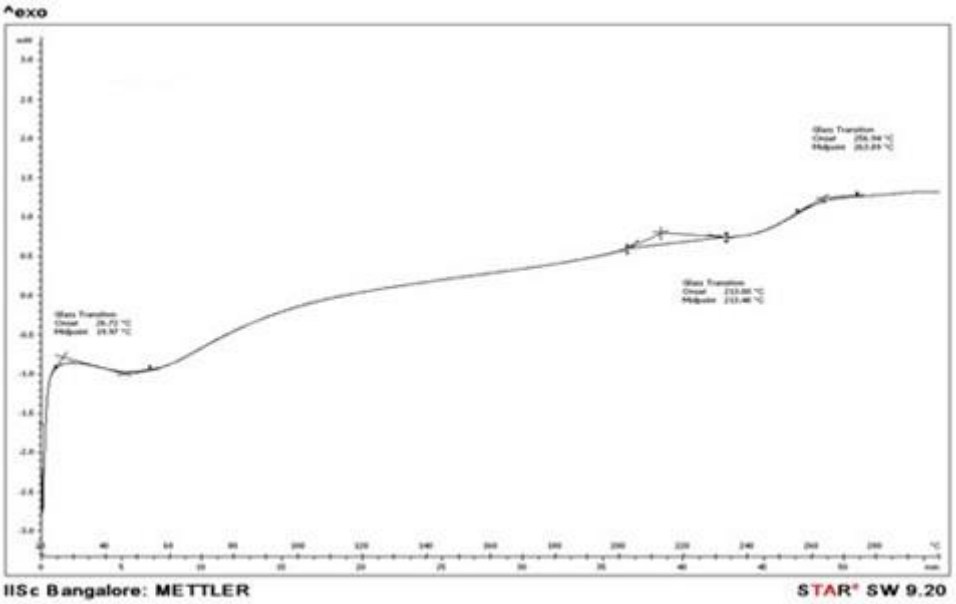

Figure 17: DSC Thermogram of Lornoxicam beads

In Vitro Dissolution Studies:

The concentration of eudraagit was optimized. With the increase in the concentration of the Eudragit S100 and Eudragit L100 in the coating solution there isconsiderable increase in the lag time for drug release, namely, when 2% Eudragit was used for coating. The lag time was observed to be 3 hrs for 1% and 6 hrs while 4% and 6% Eudragit showed lag time 8 hrs to 9 hrs, respectively. Hence, Eudragit concentration as 2% was optimized to get the desired lag time for the drug release. The bare pectin alginate beads showed immediate drug release while, when the beads are filled in the enteric gelatin capsule there was a pH dependent drug release after the dissolution of the capsule shell. Drug release was inversely related to the concentration of sodium alginate and cross-linking of beads. The decrease in drugrelease resulted in increase in sodium alginate concentration and increasing the cross-linking time. The effective sustained release was obtained from different formulations. The uncoated formulations F1, F4, P6, and F7 showed much sustained drug release than formulation F2, F3, and F5. Moreover, amongst this formulation F6 and F7 gave more sustained drug release due to the formation of the rigid gel structure dual with $AlCl_3$ which reduces the drug release from gel matrix of beads, so overcome the burst release of drug from the pectin alginate beads by formulating the dual cross- linked beads.

The uncoated formulation F2, F3, and F5 gave burst release in 2 hrs after the swelling of beads. In the case of enteric-coated formulation F1 and F4 formulation depicted 6 hr lag time and F3, F4, and F5 formulation show lag time 5 hr because of lesser concentration of sodium alginate but the formulation F6 and F7 demonstrated 7 hrs to 8 hrs lag time due to dual cross-linking with $AlCl_3$. In case of dual cross-linking rigid gel structure is formed which reduce the diffusion of drug from beads and increase the lag time of formulation. The *invitro* performance of Lornoxicam beads showed prolonged and controlled release of Lornoxicam with 7 hrs lag time.

Table 7: Optimization of Coating Concentration [Eudragit S100 & Eudragit L100 (1: 2)]

S. No.	Time (hr.)	F1 (1%)	F1 (2%)	F1 (3%)	F1 (4%)
1	1	2.13	1.11	0.97	0.31
2	2	9.44	7.25	3.24	1.21
3	3	10.88	8.86	4.11	2.77
4	4	25.63	9.91	5.78	3.97
5	5	29.88	10.34	7.10	5.60
6	6	48.94	18.71	10.44	7.14
7	7	72.13	29.81	13.73	9.32
8	8	86.44	41.35	21.74	12.81
9	10	91.44	66.90	35.97	28.44

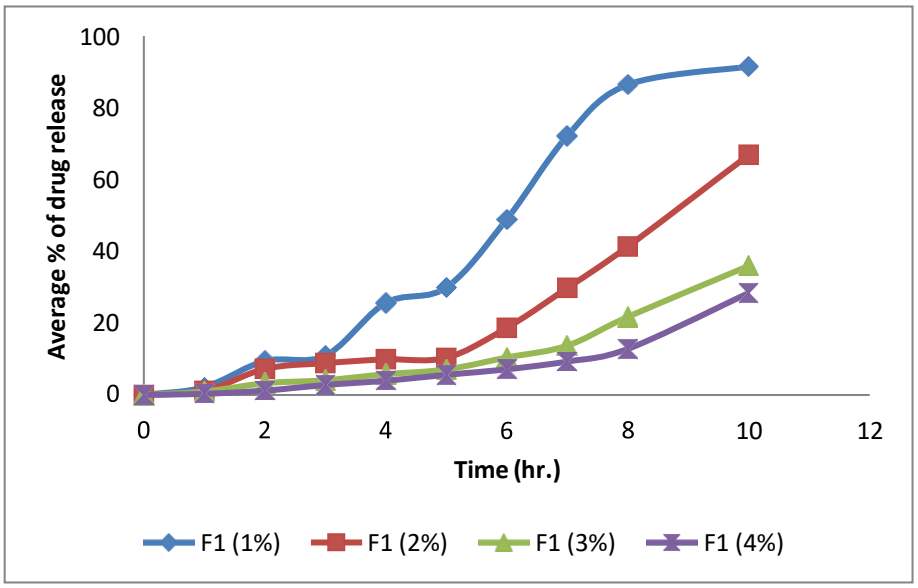

Figure 18: Optimization of Coating Concentration [Eudragit S100 &EudragitL100 (1: 2)]

Table 8: *Invitro* release from uncoated formulation of Lornoxicam beads (F1-F4).

Sl. No.	Time (hr)	% Cumulative drug release			
		F1	F2	F3	F4
1.	0	0	0	0	0
2.	1	15.11	21.28	19.15	12.25
3.	2	44.53	88.11	82.37	29.13
4.	3	61.62	94.18	87.84	52.35
5.	4	70.24	97.29	89.68	68.18
6.	5	77.14	98.48	89.48	93.55
7.	6	82.71	98.98	91.75	95.52
8.	7	89.34	99.25	95.63	96.76
9.	8	94.62	99.72	97.37	96.43
10.	10	99.67	98.94	98.55	98.27

Table 9: *In-vitro* release from uncoated formulation of Lornoxicam beads (F5-F7).

Sl. No.	Time (hr)	% Cumulative drug release			
		F5	F6	F7	F8
1.	0	0	0	0	0
2.	1	37.21	8.18	7.33	7.13
3.	2	76.64	12.74	10.66	11.26
4.	3	83.95	38.29	32.15	28.55
5.	4	85.23	62.35	58.56	48.53
6.	5	88.63	83.72	79.57	69.48
7.	6	91.84	89.53	85.45	80.93
8.	7	93.3	92.18	90.46	85.19
9.	8	94.84	93.86	91.86	89.76
10.	10	99.97	99.14	93.63	96.45

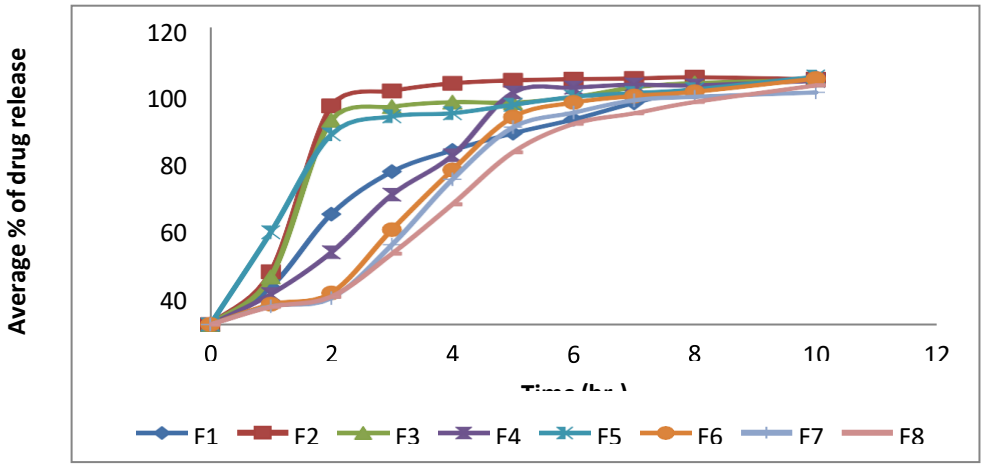

Figure 19: Comparative *In Vitro* Release Profile of Lornoxicam Beads

Table 10: In Vitro Release from Coated Formulation of Lornoxicam Beads

S. No.	Time (hr.)	% Cumulative drug release						
		F1	F2	F3	F4	F5	F6	F7
1.	0	1.13	2.18	1.33	1.2	1.2	1.66	1.75
2.	1	6.25	6.72	7.26	4.75	6.75	7.13	3.53
3.	2	7.88	8.18	7.67	5.34	7.83	8.15	8.23
4.	3	7.93	8.44	8.56	7.14	9.24	8.66	9.57
5.	4	9.36	10.65	11.72	10.86	11.73	12.09	11.19
6.	5	18.73	72.13	43.84	31.24	41.55	19.74	13.45
7.	6	29.84	87.17	56.26	41.46	82.16	46.75	31.58
8.	7	41.35	90.48	72.66	59.43	95.74	56.47	44.23
9.	8	66.92	92.64	88.88	70.14	99.92	69.35	63.89
10.	10	99.16	99.93	99.88	99.85	99.95	99.88	99.92

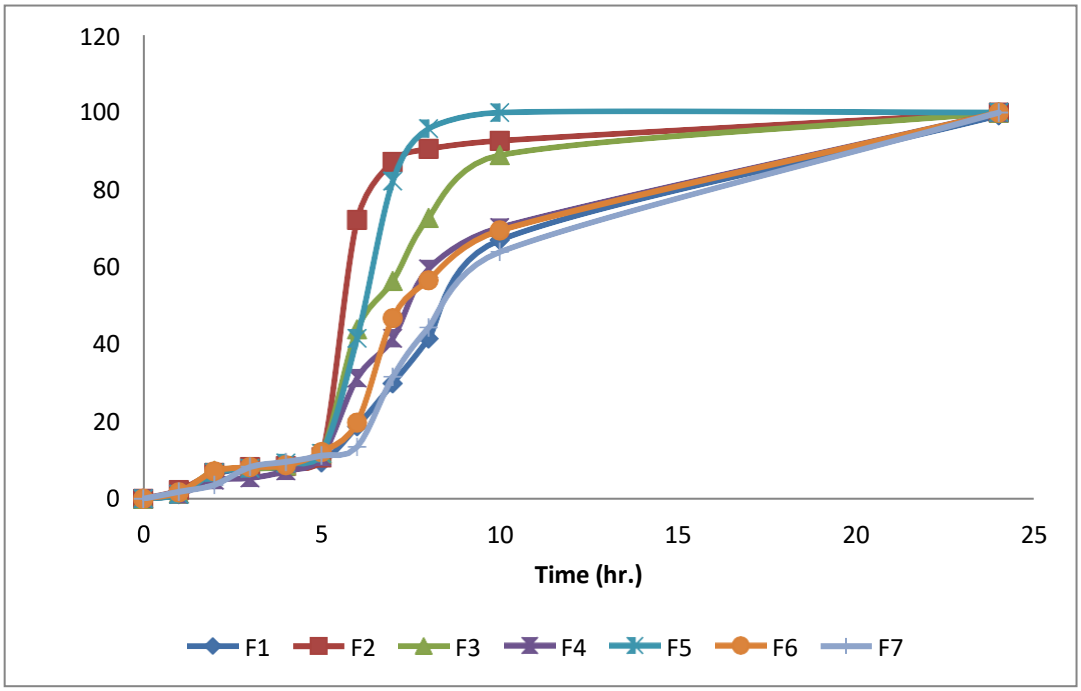

Figure 20: Comparative *InVitro* Release Profile of Lornoxicam coated Bead

CONCLUSION:

The present study was aimed at exploring the feasibility of time and pH dependent dual cross-linked pulsatile beads-based drug delivery system of Lornoxicam to modulate the drug level in synchrony with the circadian rhythm of arthritis. In the present research work, the researcher had at tempted to develop novel dosage forms by using chrono pharmaceutical approach. A dual cross-linked pulsatile dosage form with 7-8 hrs lag time, taken at bedtime and start of drug release in early morning hours, can prevent the early morning stiffness and the sharp increase of pain in arthritic patients during the early morning hours and further maintenance for a longer period of time, thereby reducing the frequency of administration and hence assure patient compliance.

From the above experimental results it may be summarized as:

1. The Pre formulation studies like pH, melting point, solubility UV-analysis and FTIR study of Lornoxicam were found to comply with of facial standards.
2. The FTIR Spectra revealed that there was no interaction between the polymers and drug. Polymers used were compatible with Lornoxicam.
3. Surface smoothness of the Lornoxicam beads was enhanced by increase in the polymer concentration, which was confirmed by SEM.
4. Increase in amount of polymer increased the particle size and drug entrapment efficiency of the Lornoxicam beads.
5. Invitro drug release of beads showed biphasic release pattern with initial minimum burst release effect, which may be attributed to the Lornoxicam loaded on to surface of the particles.

ACKNOWLEDGEMENT:

By the grace and mercy of almighty GOD, the most beneficent and merciful, this thesis entitled "**DEVELOPMENT AND EVALUATION OF DUAL CROSS LINKED PULSATILE BEADS FOR CHRONOTHERAPY OF RHEUMATOID ARTHRITIS**" has been a final shape. I extend my sincere thanks **Dr. Sunil Prakash chaturvedi, Professor, Rajeev Gandhi college of pharmacy, Bhopal, MP** for his timely help throughout the project as well as providing instrumental facilities for carrying out of my work. Teacher is a guide, philosopher and friend, which I could experience in my respected dear and esteemed guide. I consider myself the luckiest to work under the guidance of **Mr. Sanjay kumar Dhaker, Associate Professor, Department of Pharmaceutics, Nri institute of pharmaceutical sciences, Bhopal, M.P.** I take this opportunity to express my deep gratitude, sincere and heart-ful thanks to my reverend guide. It provides me pleasure to convey my graduate to all those who have directly or indirectly contributed to make this work a success.

REFERENCES:

1. Terrific Arthritis Gadgets - Arthritis Center - Everyday Health". EverydayHealth.com. Retrieved 2019-03-08.
2. R. Vidhyalakshmi, 1R. Bhakyaraj and 2R.S. Subhasree Encapsulation "The Future of Probiotics"-A Review Advances in Biological Res 2009; 3(3-4): 96-103.
3. 3-D Printing Offers Helping Hand to People with Arthritis". OrthoFeed. 2018-12-15. Retrieved 2019-03-08.
4. Abanesh kumar Bansal and Vishal Pande, "Development and Evaluation of Dual Cross-Linked Pulsatile Beads for Chronotherapy of Rheumatoid Arthritis," Journal of Pharmaceutics, vol. 2013, Article ID 906178, 8 pages.
5. Abdul B, John B. Perspectives on Colonic Drug Delivery. Business Briefing.Pharma tech 2003: 185-90.
6. Ali S, Lally EV (November 2009). "Treatment failure gout". Medicine and Health, Rhode Island. 92 (11): 369–71.
7. Alick Cameron, "Musgrave, William (1655–1721)", Oxford Dictionary of National Biography, Oxford University Press, Sept 2004.
8. AmitavaGhosh, Udaya Kumar Nayak, Prasant Rout, Tanusree Nag, Partha Roy. Preparation, Evaluation and in vitro- in vivo Correlation (IVIVC) study of Lamivudine Loaded Microspheres. Res J Pharm and Tech 2008; 1(4): 353-356.
9. Arthritis and Rheumatic Diseases". NIAMS. October 2014. Archived fromthe original on 4 October 2016. Retrieved 14 June 2019.
10. Arthritis Basics. CDC. May 9, 2016. Archived from the original on 17 September 2016. Retrieved 14 June 2019.
11. Arthritis CDC. July 22, 2015. Archived from the original on 22 September 2016. Retrieved 14 June 2019.
12. Arthritis Drugs". arthritistoday.org. Archived from the original on 22 July2010. Retrieved July 5, 2010.
13. Arthritis History Archived 2010-01-30 at the Wayback Machine Medical News. Retrived on12 June 2019.
14. Arthritis Types. CDC. June 22, 2016. Archived from the original on 14 September 2016.

Retrieved 14 June2019.
15. Arthritis: An Overview. OrthoInfo. October 2007. Archived from the original on 19 September 2016. Retrieved 14 June2019.
16. Athanasiou, Kyriacos A.; Darling, Eric M.; Hu, Jerry C.; DuRaine, GraysonD.; Reddi, A. Hari (2013). Articular Cartilage. CRC Press. p. 105.
17. Bakan JA, Anderson JA. Microencapsulation. In, Theory and Practice ofIndustrial Pharmacy, 2nd ed. Lea &Febiger, Philadephia; 1967: 420.
18. Becker, Michael A. (2005). Arthritis and Allied Conditions: A textbook of Rheumatology edition 15. Lippincott Williams & Wilkins. pp. 2303–2339.
19. Bhavana V, Khopade AJ, Jain VVD, Jain NK. Oral pulsatile drug delivery.
20. The Eastern Pharmacist 1996; 39(464): 21-6.
21. Bi-Botti CY. Chronopharmaceutics: Gimmick or clinically relevant approachto drug delivery- A Review. J Control Rel 2004; 98(3): 337-53.
22. Bjorn Lemmer. The clinical relevance of chronopharmacology in therapeutics. Pharmacological Res 1996; 33(2): 107-15.