IOSR Journal Of Pharmacy *www.iosrphr.org*
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 6, Issue 3 (March 2016), PP. 23-28

Formulation and In Vitro Evaluation of Piroxicam Loaded Alginate Microspheres (Colon Specific) Using Ionotropic Gelation Technique

Kalla Madhavi[1], Dr S.Shobha Rani[2], Fathima Zahra[3].
[1]*Asst.Prof. Care College of Pharmacy, Warangal.*
[2]*Asst.Prof. CPS, IST, JNTUH, Kukatpally, Hyderabad.*
[3]*Care College of Pharmacy, Warangal.*

Abstract: *The main aim of the present work was to develop colon specific piroxicam loaded alginate microspheres intended for the treatment of rheumatoid arthritis. A multiparticulate system with a combined property of pH sensitive and biodegradability was developed. Piroxicam microspheres were prepared by using ionotropic gelation technique and coated by using coacervation phase separation method using different ratios of eudragit S100 which is a pH sensitive polymer. Both coated and uncoated piroxicam microspheres were evaluated for % yield, entrapment efficiency, particle size, flow property, in vitro percent drug release and release kinetics. Entrapment efficiency of the prepared microspheres was above 85%. Uncoated microspheres showed a delay drug release for about 10hr where as in case of eudragit S100 coated microspheres showed a delayed drug release for about 24hr in a pH medium mimicking the condition of GIT.*

Keywords: *Alginate microspheres, Coacervation phase separation, Colon specific microspheres, Ionotropic gelation technique, Piroxicam microspheres.*

I. INTRODUCTION

Rheumatoid arthritis (RA) is a very common chronic inflammatory auto immune disease characterized by an inflammation of the synovial joints, associated with significant morbidity including disability and pain. It shows different pathophysiology and follows circadian rhythms. Chronopharmacotherapy, the drug regime based on circadian rhythms would be more convenient [1]. Piroxicam is a potent non steroidal anti inflammatory drug, it shows analgesic and anti inflammatory properties and relieves pain within 30-60min of administration. As NSAIDS have severe side effects of GIT and so as to avoid GIT side effects, drug targeting is selected. Microspheres are well accepted technique to achieve sustain release, improve bioavailability, reduce adverse effects and prolong drug release and thus reduce dosing frequency [2, 3].

II. MATERIALS AND METHODS

Piroxicam is a gift sample from TRIDENT pvt ltd, Hyderabad, India. Sodium alginate, calcium chloride was purchased from SD fine chemicals. Eudragit S 100 was purchased from Himedia laboratories. All other materials used were of pharmaceutical grade.

2.1 Method of Preparation

Colon specific piroxicam microspheres were prepared by using ionotropic gelation method using alginate as a polymer. Formulations were prepared using 4% sodium alginate, containing required amount of piroxicam, stirred until it forms a smooth bubble less gel. The so formed alginate solution is dropped drop wise from a constant height using 22 guage needle in a $CaCl_2$ solution kept under continous stirring (solvent used is 1% glacial acetic acid).

Droplets when come in contact with $CaCl_2$, gets jellified and form microspheres, which are kept under continous stirring for about 1hr, followed by decantation. Washed several times with distilled water, freeze dried and stored in a closed container [4].

2.2 Coating of Piroxicam Microspheres

Prepared alginate piroxicam microspheres were coated with eudragit S 100 (pH dependent polymer). Prepared core microspheres were dispersed in 10ml of coating solution which was prepared by dissolving required amount of eudragit S 100 in ethanol-acetone solvent containing 0.2%w/v of span80, agitated at about 600rpm. About 50ml of hexane was added to the coating solution at a rate of 1ml/min. Stirring was continued for about 1hr to complete coating process. Coated microspheres were washed with excess amount of n-hexane, filtered and dried at room temperature [5].

Coating of microspheres was done on the basis of % cumulative drug release of uncoated microspheres.

Table: 1 Formulation of Uncoated Piroxicam Microspheres

Formulation code	Drug (mg)	Sodium alginate (%)	CaCl2 (%)	Cross linking time (min)
F1	100	2.5	4	60
F2	100	3	4	60
F3	100	3.5	4	60
F4	100	4	4	60
F5	100	4.5	4	60
F6	100	5	4	60

Table: 2 Formulations of Coated Piroxicam Microspheres

Formulation code	Drug (mg)	Sodium alginate (%)	Eudragit S 100 (mg)
F5a	100	4.5	100
F5b	100	4.5	200
F5c	100	4.5	300
F5d	100	4.5	400
F5e	100	4.5	500
F5f	100	4.5	600
F5g	100	4.5	700

2.3 Evaluation of Piroxicam Microspheres

2.3.1 Percent Yield

Percent yield of the prepared microspheres was determined using the formula:

$$\text{Percent yield} = \frac{\text{Practical value}}{\text{Theoretical value}} \times 100$$

2.3.2 Entrapment Efficiency

The filtrate obtained after the collection of microspheres by filtration, was diluted with phosphate buffer pH 7.4 and analyzed using a UV Visible spectrometer at 240nm to determine the amount of drug present [6].

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Total drug} - \text{Drug in solution}}{\text{Total drug}} \times 100$$

2.3.3 Particle Size Determination

Determination of average particle size was determined using optical microscopy in which eye piece micrometer is calibrated. Microspheres dispersed in distilled water, was spread on to a clean glass slide and size of 100 microspheres were measured by using the formula:

Size of individual particles (µm) = No. of division on eye piece × calibration factor

2.3.4 Angle of Repose

Angle of repose is defined as the maximum angle possible between the surface of pile of powder and the horizontal plane. Angle of repose of the prepared microspheres was determined using funnel method with a constant height of 2.5cm. Required amount of microspheres were allowed to flow out of the funnel orifice on to a plane paper kept on the horizontal surface, this forms a pile of microspheres on the paper. From the base circle of the pile, radius 'r' is calculated.

Angle of repose was calculated using the formula:

$\varphi = \text{Tan}^{-1}(h/r)$

Where h – height of the pile (2.5cm)

r – Radius of the pile.

2.3.5 Drug Polymer Interaction Study

An FTIR spectrum was done in a wave length region between 4000 and 500cm-1. The spectrum of pure piroxicam and eudragit coated alginate piroxicam microspheres were determined using IR spectrometer.

2.3.6 Surface Morphology

SEM studies have been used to study particle size distribution, surface morphology, topography; texture etc. studies were carried out by using JEOL-JSM6380LA analytical scanning electron microscope. Samples of prepared microspheres were lightly sprinkled on to a double adhesive tape, which was stuck on to aluminium stub. The photomicrographs were taken with the help of SEM analyzer.

2.3.7 In Vitro Drug Release Study

In vitro drug release studies of the prepared alginate piroxicam microspheres were performed using USP type II dissolution test apparatus.

Dissolution medium: 900ml

Stirring speed: 50rpm

Temperature: $37^0C \pm 5^0C$.

These conditions were kept constant for all dissolution studies. Dissolution medium was varied according to the following sequence, in order to mimic pH conditions of GIT, pH 0.1NHCl solution for first 2hr, pH 6.8 phosphate buffer for the next 2hr and pH 7 phosphate buffer up to 24hr. 5ml of the sample was withdrawn at regular time intervals and replaced by 5ml of fresh medium. Samples were centrifuged, filtered and analyzed at ƛ max 240nm by using UV Visible spectrophotometer [6].

2.3.8 Release Kinetics

Obtained in vitro release data was fitted into different kinetic models. By comparing the r values obtained, the best fit model was selected.

2.3.9 Stability Studies

Stability studies of the optimized formulation were performed for about 3months as per ICH guidelines at three storage conditions (40^oC & 75% RH, 25^oC & 60% RH, 5^oC). Samples were analyzed at the end of 1month, 2month and 3month.

III. RESULTS AND DISCUSSION

The prepared alginate piroxicam microspheres were evaluated and the results were as follows:

3.1 Percent yield

Percent yield of the microspheres increased with the increase in polymer concentration from formulation F1 to F6. Results indicated that, as the amount of the polymer increased, attributed to an increase in viscosity which led to larger droplets and thus greater microsphere size, thereby increase in percent yield.

3.2 Entrapment efficiency

Entrapment efficiency increased as the concentration of the polymer increased i.e. from F1 to F6. As the polymer concentration increased, viscosity increased which resulted in larger droplet and lead to greater molecular size thus greater availability of active calcium binding sites in the polymeric chains and greater degree of cross linking of sodium alginate.

Table: 3 Percent Yield, Particle Size, % Drug Entrapment Efficiency and Angle of Repose of the Uncoated Microspheres

Formulation	%yield	Particle Size (µm)	% Drug Entrapment Efficiency	Angle of Repose
F1	84.4	441	96.6	$34^o1''$
F2	85.4	486	96.9	$31^o40''$
F3	88.7	501	98.01	$32^o45''$
F4	89.9	529	98.71	$29^o88''$
F5	92.5	581	98.9	$29^o68''$
F6	92.9	585	99.2	$28^o85''$

Table: 4. Percent Yield, Particle Size, % Drug Entrapment Efficiency and Angle of Repose of the Coated Microspheres

Formulation	%Yield	Particle Size (µm)	% Drug Entrapment Efficiency	Angle of Repose
F5a	93.1	601	98.1	$25^o12''$
F5b	93.4	621	98.7	$24^o28''$
F5c	93.5	648	98.6	$23^o82''$
F5d	93.6	659	98.7	$23^o1''$
F5e	93.1	701	98.7	$21^o52''$
F5f	93.2	723	98.5	$20^o12''$
F5g	93.6	736	98.6	$20^o1''$

3.3 Particle Size Analysis

Particle size distribution of each formulation was in narrow range but the mean particle size differed as the polymer concentration increased, this may be due to increase in viscosity, which led to larger droplets and thus formation of larger size microspheres.

3.4 Angle of Repose

A flow property of prepared microspheres was determined using funnel method. Formulations from F1 to F6 were passable where as from F5a to F5g showed excellent flow property, this may be due to smooth coating of eudragit.

3.5 FTIR Studies

The FTIR spectra obtained for pure drug and optimized coated formulation indicates that there is no interaction between piroxicam and all other additives used in the formulation.

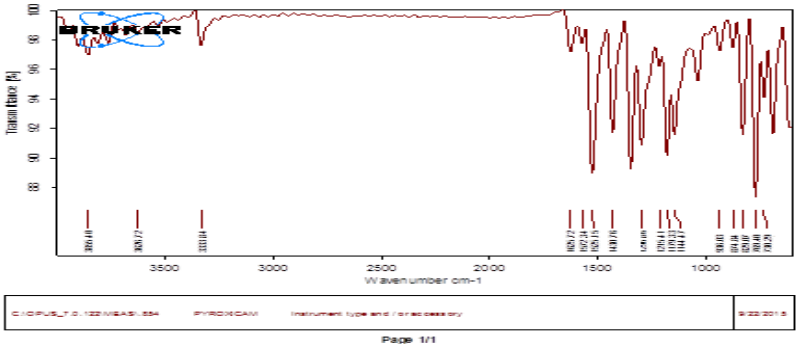

Fig 1. FTIR Spectrum of Piroxicam

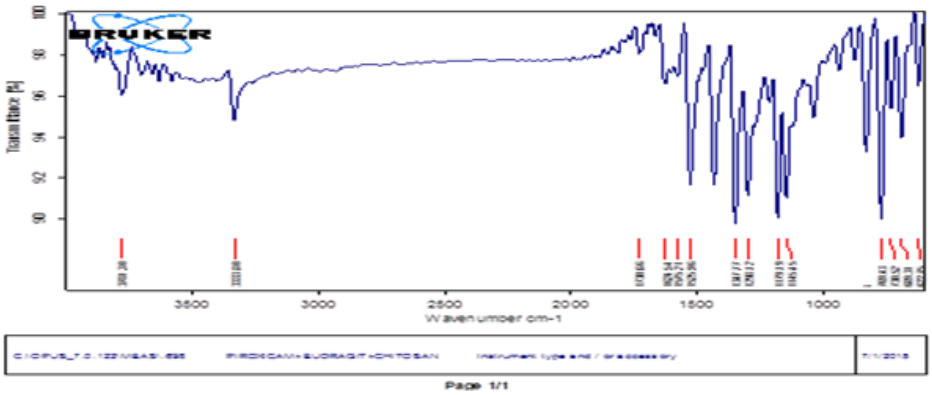

Fig 2. FTIR Spectrum of Piroxicam and Eudragit Mixture

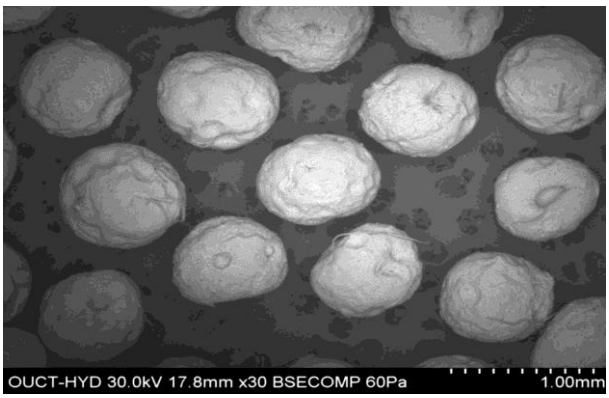

Fig 3 SEM Picture of Prepared Piroxicam Microspheres

Surface morphology was studied using SEM, studies revealed that particles ranged from oval to spherical with rough surface, this may be due to uniform dispersion of the drug in molecular level in the alginate polymer matrix.

3.6 In Vitro Release Studies

Sodium alginate was used at different concentrations i.e. 2.5, 3.0, 3.5, 4.0, 4.5% w/v. As the concentration of sodium alginate increased more sustained release was seen. In the first phase negligible amount of piroxicam was released (lag time) might be due to negligible dissociation of prepared microspheres in phosphate buffer and the drug from small pores and cracks formed. In the second phase it showed burst release which was accompanied by microsphere's disintegration. As the concentration of alginate increased availability of cross linking sites increased leading to the formation of tight junction thus lead to more sustained action [7]. Optimized formulation i.e. F5 were coated with different concentrations of eudragit S100 to prevent drug release in gastric and enteric environments.

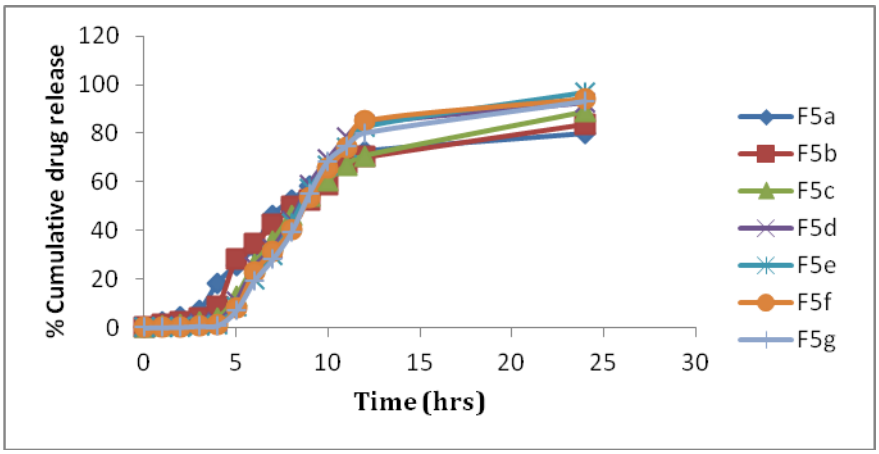

Fig 3 Graph Showing Drug Release Profile of Coated Microspheres

Eudragit coated microspheres showed no drug release in 0.1N HCl (simulated gastric fluid) up to 2hr may be due to the intactness of the applied coat. In simulated intestinal fluid media negligible drug release, and about 95% release in 24hr because of the drug encapsulated in an acid resistant polymer which prevented the release of drug in the gastric media. Analyzing the overall drug release profile of the (prepared) coated microspheres it is evident that the microspheres retained integrity up to 24hr and showed sustained release.

3.7 Study of Drug Release Kinetics

The drug release mechanism was studied by fitting the drug release data in different models i.e. zero order, first order, higuchi model, korsemeyer and peppas model. Release rate constant was calculated from the slope of the equations and correlation coefficient (R) was determined. The data revealed that it follows higuchi kinetics which confirms diffusion type drug release and that the prepared microspheres were anomalous (non fickian) release mechanism.

3.8 Accelerated Stability Studies

Optimized formulation was selected for stability studies, which were performed as per ICH guidelines at three temperatures the results were as follows.

Table 5 Accelerated Stability Studies Data

Time	% Assay
1 Month	99.2
2 Month	99.0
3 Month	98.6

Based on the results it can be indicated that there is no considerable degradation during the study period.

IV. CONCLUSION

It can be concluded from the present investigation that eudragitS100 coated alginate microspheres are promising delayed release carriers for colon specific drug delivery of piroxicam and more formulation studies are needed to design perfect controlled release formulation.

REFERENCES

[1] A. Ahmed, Hussein, Mowafaq Mohammed Ghareeb, A.Renata and Abdul hussain, Preparation and characterization of piroxicam colon targeted coated tablets, International Journal of Pharmacy and Pharmaceutical Sciences, 5(1), 2013, 338-342.

[2] Amrita Chowdhury and Hariom singh, A Review: Different Approaches of Colon Targeted Drug Delivery System, Am. J. PharmTech Res, 4(6), 2014, 105-117.

[3] Sateesh Kumar Vemula and Prabhakar Reddy, Different approaches to design and evaluation of colon specific drug delivery systems, Int.Journal of Pharmacy&Technology, 1(1), 2009, 1-35.

[4] E.Christina, Preparation of microspheres of diclofenac sodium by ionotropic gelation technique, International Journal of Pharmacy and Pharmaceutical Sciences, 5 (1), 2009,228-231.

[5] Seema Badhana, Navneet Garud and Akanksha Garud, Colon specific drug delivery of mesalamine using eudragit S 100-coated chitosan microspheres for the treatment of ulcerative colitis, International Current Pharmaceutical Journal, 2(3), 2013, 42-48.

[6] M.S. Khan, B.K.Sridhar and A. Srinatha, Development and Evaluation of pH-Dependent Micro Beads for Colon Targeting, J Pharm Sci., 72(1), 2013,18–23.

[7] B. Sree Giri Prasad, C.M. Setty, V.R.M. Gupta, D. Devanna and Rama Devi, Aceclofenac-Loaded Alginate Microspheres Prepared By Ionotropic Gelation Method: Effect of Formulation Variables on Encapsulation Efficiency, Morphology and Release Characteristics, Der Pharmacia Lettre, 3(2), 2013, 10-24.