Research Article **CODEX: IJRPJK** **ISSN: 2319 – 9563**

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: **www.ijrpns.com**

DEVELOPMENT AND EVALUATION OF SUSTAINED RELEASE ACECLOFENAC MICROSPHERES USING CHITOSAN POLYMERS AND TECHNIQUES IN MANAGEMENT OF RHEUMATOID ARTHRITIS

S. Jeganath*[1] and K. Senthilkumaran[1]

[1*]Department of Pharmaceutics, K.K.College of Pharmacy, Gerugambakkam, Chennai, Tamilnadu, India.

ABSTRACT

Microsphere formulation offers a number of advantages in therapeutics where the sustained release of drugs. Aceclofenac is the most widely used anti-inflammatory agent in the treatment of rheumatoid arthritis. The objective of the present work was to formulate chitosan microspheres containing Aceclofenac in order to provide a prolonged effect and relatively constant effective levels of these drugs in the treatment of rheumatoid arthritis. In this present study microspheres of Aceclofenac was prepared by glutaraldehyde cross-linking, using Chitosan as polymer. The preparation of microspheres by chemical cross linking method was done by using glutaraldehyde as cross linking agent by varying polymer ratio. The prepared microspheres were evaluated for entrapment efficiency, particle size, surface morphology (SEM), Fourier transform infrared spectroscopy and i*n vitro* drug release. The results were conclude that FAM-1(84.36%) can be considered as a optimized formula for sustaining the release of drug for 12 hours.

KEYWORDS

Microspheres, Aceclofenac, Chitosan, Chemical cross linking method and Evaluation.

Author for Correspondence:
S. Jeganath,
Department of Pharmaceutics,
K.K.College of Pharmacy, Gerugambakkam,
Chennai, Tamilnadu, India.

Email: jeganaths@gmail.com

INTRODUCTION

The pain is symptomatic of some form of dysfunction and resultant inflammatory processes in the body. A survey conducted for the WHO reported that one adult in five suffers from chronic non-malignant pain, which mostly occurs in the back, head, joints and limbs. More than 15 % of the world population suffers for instance from some

form of osteoarthritis, and this incidence is even higher in the elderly. As the world population is grows older, this incidence will continue to rise. The word arthritis literally means inflammation of the joints characterized by pain, swelling and redness, heat and, sometimes, structural changes. Joints can become inflamed for many reasons, but arthritis usually as one of two kinds: osteoarthritis or rheumatoid arthritis. Aceclofenac has been shown to have potent analgesic and anti-inflammatory activities, similar to Indomethacin and Diclofenac and due to its preferential cox-2 blockade it has better safety than conventional NSAIDs with respect to adverse effects on gastrointestinal and cardiovascular system.

Conventional medicine previously offered only symptomatic, temporary relief from chronic arthritic conditions. High doses of the Non-steroidal anti-inflammatory drugs (NSAIDs) are effective in reducing symptoms quickly but can cause side effects such as ulcers and gastrointestinal bleeding and NSAIDs do not stop the progression of arthritis. In the long run, some anti arthritic drugs may actually worsen the condition by accelerating joint destruction.

Microspheres are defined as homogenous, monolithic particles in the size range of about 1 - 1000 μm[1] and are widely used as drug carriers for controlled release. These systems have significant importance in biomedical applications. Administration of drugs in the form of microspheres usually improves the treatment by providing the localization of the active substance at the site of action and by prolonging release of drugs. The microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for the controlled release of drug. Some of the advantages of microspheres include patient compliance because dosing is automatic and the levels in blood are preprogrammed, targeting of the drug is possible, and the bioavailability of drug is more[2]. A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of microspheres. These materials include the polymers of natural and synthetic origin and also modified natural substances.

Recently, natural polymers such as polysaccharides and proteins have received much attention in the pharmaceutical field owing to their good biocompatibility and biodegradability. Among polysaccharides, chitosan, the deacetylated product of chitin, is one of the most useful natural polymers from the viewpoint of possible exploitation of natural resources. Chitosan is insoluble at neutral and alkaline pH values, but forms salts with inorganic and organic acids such as hydrochloric acid. Upon dissolution, the amine groups of chitosan get protonated and the resultant polymer becomes positively charged[3].

The effect of chitosan has been considered mainly because of its positive charge: however, the adsorption process could also be the result of other forces that might exist between molecules, such as hydrogen bonding or vaner waal's forces. These interactions might have a strong impact on the absorption and bioavailability of pharmaceutical compounds, especially drugs that are potent and have low water solubility[4].

MATERIAL AND METHOD

MATERIALS

Aceclofenac was purchased from Restek Pharma, Pondicherry, Chitosan from Chem Pharm Private Limited, Gurgaon. Glutaraldehyde, Span-80, Heavy Liquid paraffin, Light Liquid paraffin, Petroleum Ether, Acetone, and methanol were purchased from Paxmy, Chennai. All the chemicals and substances used were of analytical grade.

Method of preparation

Glutaraldehyde Cross-linking Method

Six formulae were prepared by taking drug: polymer ratio of 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, and

1:3. The microspheres prepared were named as FAM. A2.5% (v/v) aqueous acetic acid, required quantity of chitosan was added and dissolved. To this drug was added. This dispersed phase was added to continuous phase (125ml) consisting of light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 containing 0.5% (w/v) span 80 to form water in oil (w/o) emulsion. Stirring was continued at 2000 rpm using a 3- blade propeller stirrer. A drop-by-drop solution of measured quantity (2.5ml each) of aqueous glutaraldehyde (25%v/v) was added at 15, 30, 45 and 60 mints. Stirring was continued for 2.5 hrs to obtain microspheres, which were separated by filtration under vacuum and washed first with petroleum ether and then with distilled water to remove the adhered liquid paraffin and glutaraldehyde, respectively. The microspheres were then finally dried in a desiccator[1].

CHARACTERIZATIONOF MICROSPHERES[5, 6]

Fourier Transform Infrared Spectroscopy

The Fourier transform infra-red analysis was conducted for the structure characterization. FTIR spectra of the formulated microspheres and drug were recorded. Microspheres were taken in a KBr pellet using BOMEN MB SERIES FTIR instrument. Approximately 5mg of samples were mixed with 50mg of spectroscopic grade KBr, samples were scanned in the IR range from 500 to 3500 cm^{-1}, with a resolution of 4 cm^{-1}.

Determination of drug loading in microspheres

The loading of Aceclofenac in microspheres was estimated by using the formula:-

$$L = \frac{Qm}{Wm} \times 100$$

Where,

L = percentage of drug loading in the microspheres

Wm = weight of microspheres in grams

Qm = quantity of Aceclofenac present in Wm grams of microspheres.

Determination of entrapment efficiency of the microspheres

The amount of Aceclofenac encapsulated in to the Microspheres was determined by using the formula:-

$$E = \frac{Qp}{Qt} \times 100$$

Where,

E = percentage of entrapment efficiency of microspheres.

Qt = percentage of drug loaded in the microspheres.

Qt = quantity of drug added for loading (gms).

Determination of Mean particle size of microspheres

Particle size determination of microspheres was carried out by optical microscopy. A minute quantity of dried microspheres was suspended in glycerin and the particle size of 100 microspheres was determined in each batch and the mean particle size was calculated.

Scanning electron microscopy (SEM)

For the external morphology studies, air dried particles were visualized using scanning electron microscopy (SEM Jeol JSM-6400, JAPAN) operating at 20kv. The samples were mounted on a metal stub with double adhesive tape and coated with platinum/palladium alloy under vaccum.

***In vitro* drug release studies**

The *in vitro* drug release studies were conducted in pH 7.4 buffer for 12 hours using USP XXIII, type-II dissolution apparatus under sink conditions. Accurately weighed samples of the microspheres were added to dissolution medium kept at 37°C ±5°C. At preset time intervals aliquots were withdrawn and replaced by an equal volume of dissolution medium to maintain constant volume. After suitable dilution, the samples were analyzed spectrophotometrically at 275 nm[7].

RESULTS

Pre formulation studies

Compatability studies (Fourier Transform Infrared Spectroscopic studies)

The fourier transform infra - red analysis was conducted for the surface structure characterization. FTIR spectrum of the formulation of microspheres, pure drug and polymers was recorded. Microspheres were taken in a KBr pellet using BOMEN MB SERIES FTIR Instrument. The Fourier Transform Infrared Spectroscopy study reveals that there is no interaction between the polymer and drug. Then all the functional groups found in the IR spectrum of pure drug, polymers and microspheres.

Percentage of drug entrapment efficiency

The percentage entrapments for all formulations were given in Table No. 2. The results show that the entrapment efficiency of FAM-1 was 43.2% followed by FAM-2 of 40.7% and FAM -6 by 27.2% and considered to be high when compared to all other formulations.

Mean particle size

Particle size was determined by optical microscopy and the average particle size for all batches of microspheres was given in Table No.3. All batches of microspheres were prepared by keeping the drug amount and solvent volume constant. The results indicate that the mean particle size increases with increase in polymer concentration. These results showed that the viscosity of inner phase is an important factor which determines the particle size of microspheres. As the viscosity increases particle size increases.

***In vitro* drug release studies**

The *in vitro* release profiles for batch FAM-1, FAM-2, FAM-3, FAM-4, FAM-5 FAM-6 were conducted in pH 7.4 buffer for 12 hours and the results was shown in Table No.4.

The *in vitro* release profile of the best formulation **FAM-6** was shown in Table no.4 and it shows 93.56% of sustained drug release at the end 12 hours when compared to all other formulations. The comparison of *in vitro* release of all other formulations with the best formulation **FAM-6** was shown in Figure No.1.

Scanning Electron Microscopy

The particle size of the optimized formulation **FAM -6** was carried out by scanning electron microscopy. The SEM photographs in different magnifications were shown in Figure No.2. The particle size of the formulation ranging from 100 to 300µm which confirms the formulation was having desired particle size for microspheres.

Table No.1 Formula of Aceclofenac microspheres by using various proportions of Chitosan polymer

S. No	Ingredients	Batches of FAM microspheres prepared					
		FAM-1	FAM-2	FAM -3	FAM -4	FAM-5	FAM-6
1	Drug (Aceclofenac)	500mg	500mg	500mg	500mg	500mg	500mg
2	Chitosan	250mg	500mg	750mg	1000mg	1250mg	15000mg
3	Heavy liquid paraffin	62.5ml	62.5ml	62.5ml	62.5ml	62.5ml	62.5ml
4	Light liquid paraffin	62.5ml	62.5ml	62.5ml	62.5ml	62.5ml	62.5ml
5	Span- 80	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
6	Glutaraldehyde	10ml	10ml	10ml	10ml	10ml	10ml

Table No.2: Percentage of drug entrapment efficiency of Aceclofenac microspheres

S.No	Formulation	Drug: Polymer	% entrapment Efficiency
1	FAM-1	1:0.5	43.2
2	FAM-2	1:1	40.7
3	FAM-3	1:1.5	38.3
4	FAM-4	1:2	34.5
5	FAM-5	1:2.5	27.8
6	FAM-6	1:3	27.2

Table No.3: Mean particle size of FAM-6 formulation

S.No	Formulation code	Size range (µm)	Mean of size range (µm), d
1	FAM-6	0-99	48.6
		100-199	148.9
		200-299	249.4
		300-399	348.3
		400-499	448.7

The arithmetic mean of particle size = 242.495µ

Table No.4: *In vitro* release profiles of Aceclofenac Chitosan microspheres in pH 7.4

S.No	Time (hours)	% of Drug Release					
		FAM -1	FAM -2	FAM -3	FAM -4	FAM -5	FAM -6
1	0	0.00	0.00	0.00	0.00	0.00	0.00
2	1	5.02	6.24	7.35	8.12	10.54	12.87
3	2	8.17	9.32	12.21	15.34	21.03	24.23
4	3	12.54	13.24	15.47	24.42	28.17	34.54
5	4	15.54	17.84	21.35	30.25	34.02	41.32
6	5	20.76	23.52	38.41	40.24	41.65	50.23
7	6	24.54	27.85	43.25	45.74	49.35	56.12
8	7	32.24	36.74	48.62	52.24	55.32	60.53
9	8	35.42	39.81	52.54	59.45	61.24	67.13
10	9	45.52	48.24	59.32	65.24	69.32	73.57
11	10	50.25	54.84	64.53	72.14	78.97	83.29
12	11	58.36	63.47	70.24	78.32	82.38	89.25
13	12	65.58	71.69	77.43	84.32	88.23	93.56

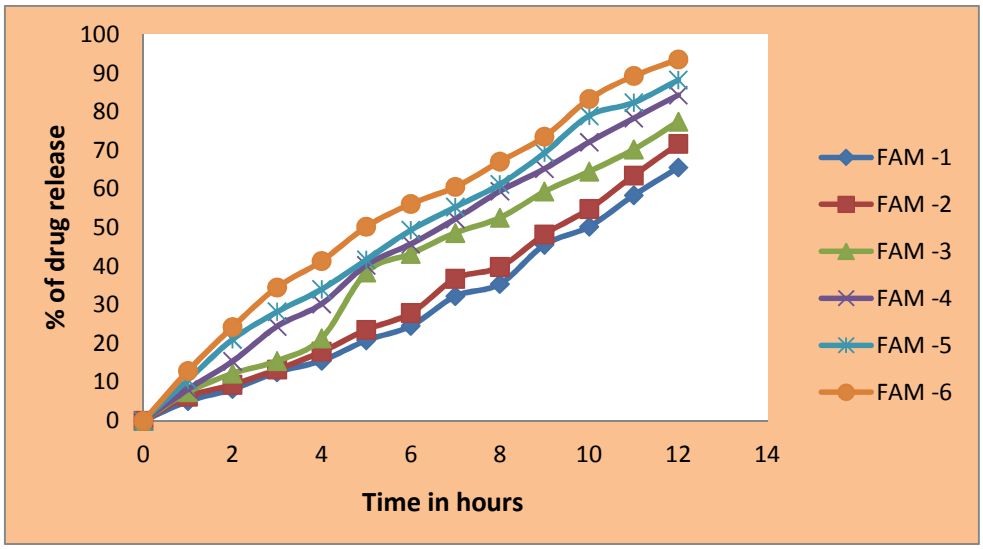

Figure No.1: *In vitro* release studies of Aceclofenac Chitosan microspheres in phosphate buffer (pH 7.4)

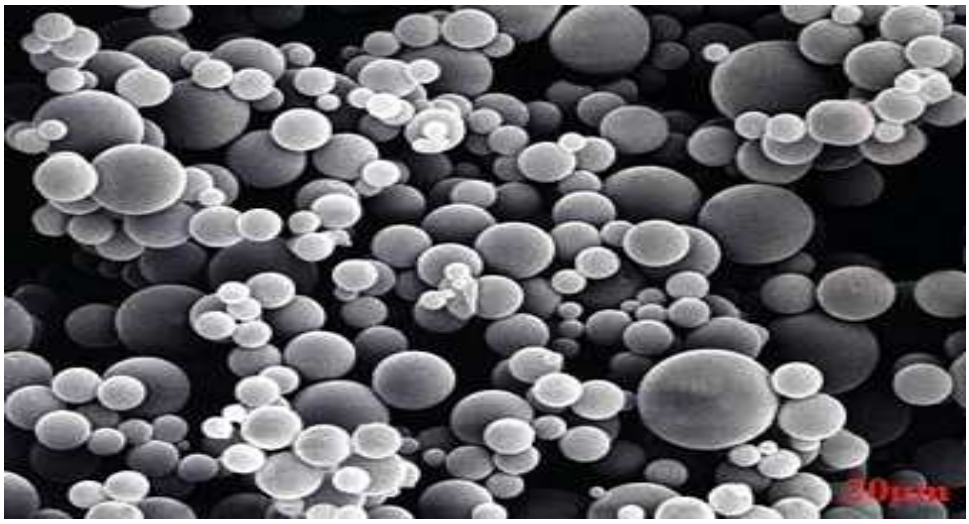

Figure No.2: SEM Photograph of FAM - 6

CONCLUSION

Novel drug delivery systems are becoming one of the most important fields in the modern pharmaceuticals. In the present study an attempt has been made to develop Acelclofenac loaded microspheres by with varying concentrations of chitosan. The *in vitro* studies show a prolonged release of Aceclofenac over 12 hours. The results conclude that **FAM-6** can be considered as an optimized formula for sustaining the release of drug for over 12 hours and the formulation can be considered as best alternate to sustained release tablets for the treatment of rheumatoid arthritis and can be best used with minimal or without any major side effects associated with sustained release tablets.

ACKNOWLEDGEMENT

The authors are sincerely thanks to the K.K.College of Pharmacy, Gerugambakkam, Chennai, Tamilnadu, India for providing the facilities to complete this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Acikgoz G M, Mahrous M, Al-dardan M. Preparation and Evaluation of sustained release crosslinked chitosan microspheres containing phenobarbitone, *J. Microencapsulation,* 15(3), 1998, 373-382.
2. Desai S and Bolton S. A floating controlled-release drug delivery system: *in vitro-in vivo* evaluation, *Pharm res,* 10(9), 1993, 1321-1325.
3. Hua zhang, Ibrahim A Alsarra and Steven H Neau. An *in vitro* evaluation of a chitosan-containing multiparticulate system for macromolecule delivery to the colon, *International journal of pharmaceutics,* 239(1-2), 2002, 197-205.
4. Patil S S and Kasture P V. Design and Evaluation of biodegradable poly l-lactide microspheres of aceclofenac, *J. Pharm. Sci*, 6(1), 2007, 24-28.
5. Senthil kumar S K, Jayakar B, Kavimani S. *In vivo* evaluation of gastro retentive floating microsphere containing rebeprazole sodium, *international journal of biological and pharmaceutical research*, 2(2), 2011, 80-84.
6. Roy S, Panapalia S G, Nandy B C, Rai V K, Tyagi L K, Dey S, Meena K C. Effect of method of preparation on chitosan microspheres of mefenamic acid, *International journal of pharmaceutical sciences and drug research*, 1(1), 2009, 36-42.
7. Sakchai Wittaya-areekul, Jittiporn Kruenate, Chureerat Prahsarn. Preparation and *In vitro* evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone, *International journal of pharmaceutics*, 312(1-2), 2006, 113-118.

Please cite this article in press as: Jeganath S and Senthilkumaran K. Development and evaluation of sustained release aceclofenac microspheres using chitosan polymers and techniques in management of rheumatoid arthritis, *International Journal of Research in Pharmaceutical and Nano* sciences, 1(1), 2012, 27-34.