Studies on Preparation and Evaluation of Biodegradable Poly (Lactide-Co-Glycolide) Microsphere of Aceclofenac

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ABSTRACT

After successful development of drug entity, design of dosage form then plays a very important role. Aceclofenac, a novel NSAID is indicated for the symptomatic treatment of pain and inflammation. The mean plasma elimination half-life is 4 hours. The side effects are dyspepsia, abdominal pain, diarrhea, nausea, dizziness, flatulence, gastritis, constipation, etc. The main aim of this research work was to reduce the dosing frequency and adverse effects by formulating controlled-delivery system in the form of biodegradable microsphere. Poly (lactide-co-glycolide) was used as biodegradable microsphere. Solvent evaporation method was used for the preparation of microsphere. The SEM photograph showed that the microspheres were spherical in shape. Average particle size was 295 µm. The drug entrapment efficiency was 25%. Release study of drug from microsphere showed 90 % release in 10 hr. and about 40% drug was released in the first 30 min. Microsphere was stable at room temperature. The IR and DSC study showed no interaction of drug with polymer and no degradation during preparation of microsphere was observed.

Key words: Poly (Lactide-Co-Glycolide) (PLGA), Microsphere, Aceclofenac, Biodegradable, Solvent Evaporation Method

INTRODUCTION

After successful development of drug entity, design of dosage form then plays a very important role. The development of drug delivery systems involves an interdisciplinary approach, involving contributions from the fields of chemistry, material science, engineering, pharmacology and other biological sciences. A controlled-release drug delivery system should be able to achieve optimum therapeutic drug concentration in the blood with minimum fluctuation, to predict and reproduce release rates for extended duration, to enhance activity duration of short half-life drugs, to eliminate side effects, to reduce frequent dosing and wastage of drug and to optimize therapy and better patient compliance. Drug delivery systems

involving the use of polymers are most widely studied and biocompatible polymers have become the focus of such research. Polymers have long been used in experimental drug delivery systems to provide controlled release of small organic molecules (Folkman and Long, 1964) and macromolecules (Langer and Folkman, 1976).

Aceclofenac (Martindale, 1993; Maryadele and Smith, 1996; British National Formulary, 2001; European Pharmacopoeia, 2002) a phenyl acetic acid derivative (2-{(2, 6-dichlorophenyl) amino} Phenylacetooxyacetic acid), is a novel NSAID indicated for the symptomatic treatment of pain and inflammation. Aceclofenac directly blocks PGE2 secretion at the site of inflammation by inhibiting IL-Beta and TNF in the inflammatory cells. It stimulates the synthesis of the extra cellular matrix of human articular cartilages and inhibits neutrophil adhesion and accumulation at the inflammatory site in the early phase and thus blocks the pro-inflammatory actions of neutrophils. Recommended dose is 200 mg daily in divided doses. The mean plasma elimination half-life is 4 hours. The side effects are dyspepsia, abdominal pain, diarrhea, nausea, dizziness, flatulence, gastritis, constipation, vomitting, ulcerative stomatitis, pruritus, rash, dermatitis, etc. To reduce the dosing frequency and adverse effects for prolonged treatment, it is needed to formulate in long-acting dosage form.

For fabrication of microspheres, large number of coating materials has been used. In the present study, Poly (lactide-co-glycolide) (PLGA) was used as polymer for the preparation of microsphere.

MATERIALS AND METHODS

Aceclofenac was supplied by Emcure Pharmaceuticals, Pune, as a gift sample; PLGA was supplied by Boehringer Ingelheim Pharma GmbH & Co. KG, also as a gift sample; other chemicals were purchased.

Preparation of Aceclofenac microsphere using PLGA

Solvent evaporation technique was used. (Hutchinson and Furr, 1990). PLGA was dissolved in methylene chloride (10ml). The PVA was dissolved in water. Aceclofenac was dissolved in PLGA solution. Then added drug-PLGA solution drop- wise in 50 ml of 0.5% aqueous PVA solution with stirring. Emulsion was formed. The temperature was increased to 45°C and stirred for 1.5 hr to evaporate the solvent. The solid microspheres were obtained by centrifugation and filtration. Spherical microspheres of uniform size were formed. The factorial design and batches prepared are shown in Tables 1 and 2, respectively.

Table 1. Variables and Their Levels for Factorial Design of Aceclofenac - PLGA Microsphere.

VARIABLES	LEVELS		
	LOWER (-1)	MIDDLE (0)	UPPER (+1)
X1-Amount of Aceclofenac	0.5	1.0	1.5
X2-Amount of PLGA	0.5	1.0	1.5

Table 2. Batches for Aceclofenac - PLGA Microsphere.

Batches	X1	X2
A-PLGA-1	-1	-1
A-PLGA -2	-1	0
A-PLGA-3	-1	+1
A-PLGA-4	0	-1
A-PLGA-5	0	0
A-PLGA-6	0	+1
A-PLGA-7	+1	-1
A-PLGA-8	+1	0
A-PLGA-9	+1	+1

The following methods were used for characterization of microspheres.

1. Percent yield:

The percent yield of each batch of microsphere was obtained on weight basis with respect to the weight of starting material.

2. Particle size and size distribution:

a. Sieve analysis (Lachman et al., 1987; Martin, 2001)

The microspheres (10gm) were placed on the top of a series of six standard stainless steel sieves in the range of 150 to 600 μm, stacked from bottom to top in ascending order of aperture size. The sieves were mounted on mechanical shaker (Gyratory type) and shaken for 15 min. The weight of material on each sieve was recorded and average particle size and the size distribution were determined.

The average particle size was determined by using the following equation

$$d_{ave} = \frac{\text{wt. size}}{100}$$

b. Scanning electron microscopy

Microspheres were coated with a thin gold-palladium layer by sputter coater unit (VG-Microtech, UK) and were analyzed with a Cambridge Stereoscan S 120 Scanning Electron Microscope (Cambridge, UK), operated at an acceleration voltage of 10 kV.

3. IR Spectroscopic study

Infra Red spectrum of drug, polymer and formulation were recorded by using Jasco FTIR-410 by KBr disc method.

4. Drug entrapment efficiency

Exactly 50 mg of microsphere were weighed and added to respective solvent and sonicated for 2 hours, filtered the resultant solution through Whatman Filter No.41. Absorbance of the resultant solution was recorded by UV-Vis Spectrophotometer (Jasco V-550) at predetermined maxima of concerned drug.

The concentration of drug extracted was determined from the calibration plot.

The drug entrapment efficiency of the microsphere was calculated by the following equation:

$$\frac{\text{Drug content of the microsphere}}{\text{Initial drug loading}} \text{ X } 100$$

5. In vitro drug release (Lachman et al., 1987)

Drug release studies were performed, using USP Type II dissolution test apparatus (Tab Machines, Mumbai, India). Weighed quantity of microspheres were wrapped in parchment paper and placed in the dissolution vessel containing 900ml of phosphate buffer pH 7.4, maintained at 37±0.2°C and stirred at 100 RPM.

Aliquots of the sample (5 ml) at different time intervals were withdrawn and filtered using a Whatman Filter No.41. Any residue obtained on the filter paper was added to the dissolution vessel while replacing the 5ml of dissolution medium.

Absorbance of the resultant solution was recorded by UV-Vis Spectrophotometer (Jasco V-550) at predetermined maxima of concerned drug. The concentration of the drug released was determined from the standard calibration plot.

6. In vivo testing (Lachman et al., 1987)

Female white rabbits weighing 2.5 to 3.0 kg were used. The required dose of drug based on the body weight was given orally. The drug microsphere suspension was administered in 3 ml volume, using a catheter. The blood samples (2 ml) were withdrawn at specified time intervals. The plasma was separated by centrifugation and analyzed for drug content by HPLC method, using Jasco HPLC Japan (Jasco 970 Detector and 980 Pump).

7. Thermal analysis

The DSC thermogram was obtained by using DuPont 2100 v 4.1c DSC. The samples were placed in copper pan and heated at a constant rate of 20°C/min over a temperature range of 30°C to 400°C under the nitrogen purging.

8. Stability testing

Microspheres were hermetically sealed in tubes and stored at 5°C, 25°C and 40°C. At the end of each week, one tube was used for evaluation. The study was carried out for six weeks. The microspheres were evaluated for physical appearance and drug content.

RESULTS AND DISCUSSION

PLGA microspheres containing aceclofenac were prepared by solvent evaporation method. Methylene chloride was selected as solvent because PLGA and the drug are freely soluble in it. The method is quick and easy.

% Yield –

The yield obtained from all batches was good. Mean percent yield was 78%. The effect of drug and polymer concentration was studied. Both increased the yield at moderate level.

Particle Size Analysis of Aceclofenac - PLGA microsphere

Microspheres were spherical, monolithic particles with no visible major surface irregularity. A microsphere formed at higher polymer concentration was much larger and denser than that formed at lower concentration. This, in turn, would increase the retention of the drug in the microspheres. The average particle size of aceclofenac-PLGA microsphere was 295 μm.

Scanning Electron Microscopy

The resultant microspheres were spherical in shape when observed under the microscope. The surface topography was studied by scanning electron microscopic photograph. It shows smooth surface.

Figure 1 represents the SEM photograph of aceclofenac-PLGA microsphere.

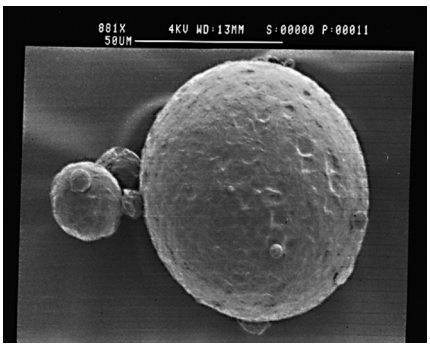

Figure 1. Scanning Electron Microphotograph of Aceclofenac-PLGA Microsphere.

IR Spectroscopic Study

The peaks of drug and polymer were observed at identical wave number. It showed that no degradation of drug had occurred during the preparation. Presence of amino peak NH stretching at 3325 as well as at 3010 as CH stretching aromatic confirmed the presence of drug in polymer. While in polymeric peaks, i.e., 2800-2900 for aliphatic CH stretching and 1698-1780 for C=O stretching confirms the PLGA. As there was no alteration in the nature of peaks, no interaction was observed between drug and polymer.

Figure 2 shows the IR spectrum of aceclofenac-PLGA microsphere.

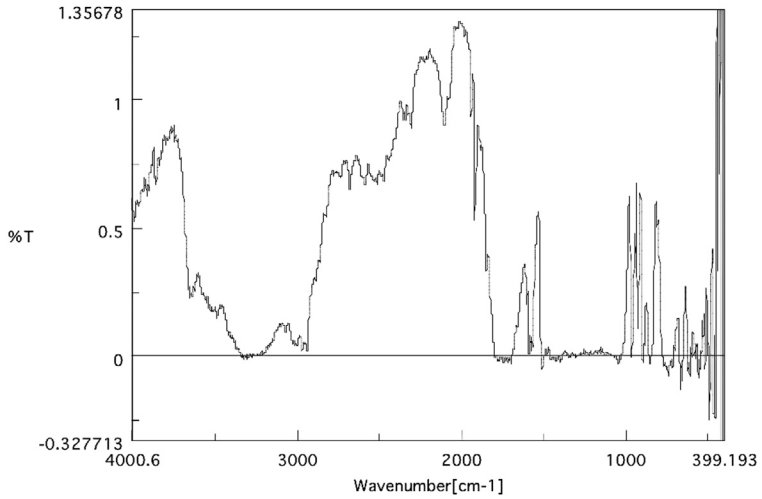

Figure 2. IR Spectrum of Aceclofenac-PLGA Microsphere.

Drug Entrapment Efficiency

Percentage drug entrapment efficiency in microspheres was found to increase with increase in drug concentration as well as polymer concentration. This might be due to higher concentration of the polymer that increased the viscosity of the medium.

Table 3 shows the entrapment efficiency of aceclofenac-PLGA microsphere.

Table 3. Drug Entrapment Efficiency in Aceclofenac-PLGA Microsphere.

Serial No.	Formulation	Drug entrapment efficiency (%)			Mean	Standard deviation
		1	2	3		
1	A-PLGA -1	20.16	23.51	22.56	22.08	1.726509
2	A-PLGA -2	23.15	26.43	24.35	24.64	1.659558
3	A-PLGA -3	25.16	27.1	23.51	25.26	1.796951
4	A-PLGA -4	23.46	26.13	23.56	24.38	1.513484
5	A-PLGA -5	26.72	24.13	25.58	25.48	1.298088
6	A-PLGA -6	26.41	25.81	26.43	26.22	0.352326
7	A-PLGA -7	24.16	23.86	24.31	24.11	0.229129
8	A-PLGA -8	27.31	25.13	26.1	26.18	1.0922
9	A-PLGA -9	27.56	26.26	25.64	26.49	0.979864

In vitro drug release

The release profiles of aceclofenac from the different formulations of the PLGA microspheres are shown in Figure 3. The data clearly indicate that the drug release can be controlled by varying the polymer concentration.

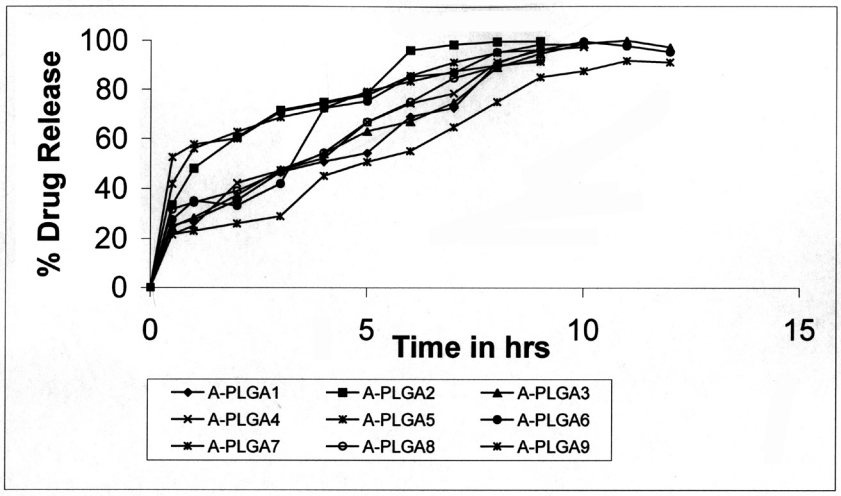

Figure 3. Drug Release Profiles from Aceclofenac - PLGA Microsphere.

The release of aceclofenac from the PLGA microsphere followed the first-order kinetics. About 10 to 40% of drug was released within 30 min. This might be due to fast dissolution of drug on the surface and more than 90% of the drug was released in 10 hours.

Table 4 shows release kinetics of aceclofenac-PLGA microsphere.

Table 4. Release Kinetics of Aceclofenac from PLGA Microspheres.

Batch no.	Zero-order		First-order		Higuchi Matrix		Peppas Equation	
	r	k	r	k	r	k	r	k
1.	0.9695	9.9952	0.9861	-0.1933	0.9529	24.9181	0.9660	21.3733
2.	0.7603	12.3096	0.9913	-0.2600	0.9831	31.9165	0.9856	36.6295
3.	0.9683	9.5095	0.9914	-0.1857	0.9749	25.0941	0.9878	21.0837
4.	0.9612	9.5994	0.9871	-0.1781	0.9838	25.4270	0.9926	19.9328
5.	0.4003	11.0356	0.9664	-0.2266	0.9172	30.4463	0.9571	47.7548
6.	0.8847	9.2889	0.9559	-0.2083	0.9762	27.2206	0.9565	24.6555
7.	0.9733	8.9875	0.9898	-0.2053	0.8915	23.0478	0.9266	15.3519
8.	0.9297	10.4246	0.9716	-0.2011	0.9710	26.3269	0.9409	27.7869
9.	0.6810	11.8377	0.9867	-0.2417	0.9685	30.7910	0.9887	40.9732

In vivo Study

The in vivo testing on white Indian rabbit was carried out. The Cmax was observed in 5 hr. Initial faster release observed and it seemed to be the prompt release of aceclofenac from microsphere. It might be due to the greater rate of elimination than the release rate of aceclofenac from microsphere after Cmax. But up to 10 hr satisfactory amount of drug found in blood. The complete elimination occurred in 48 hrs.

Figure 4 represents the in vivo release profile from aceclofenac-PLGA microsphere.

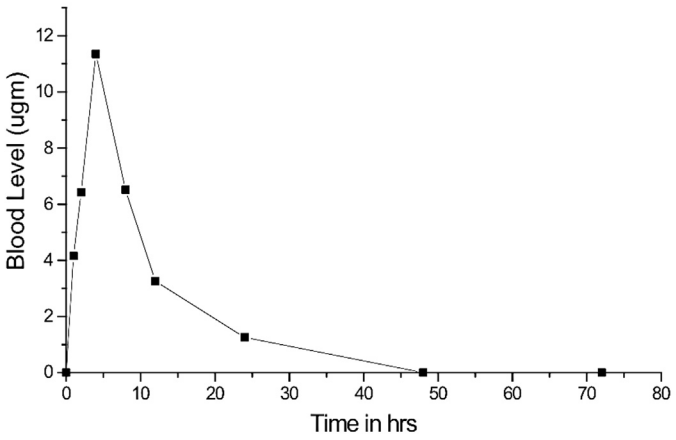

Figure 4. In vivo Release Profile of Aceclofenac-PLGA Microsphere.

DSC Study

The thermal analysis indicated good stability. The endotherm at 154°C indicated melting of aceclofenac. No interaction with polymer was observed.

Figure 5 represents the DSC thermogram of aceclofenac-PLGA microsphere.

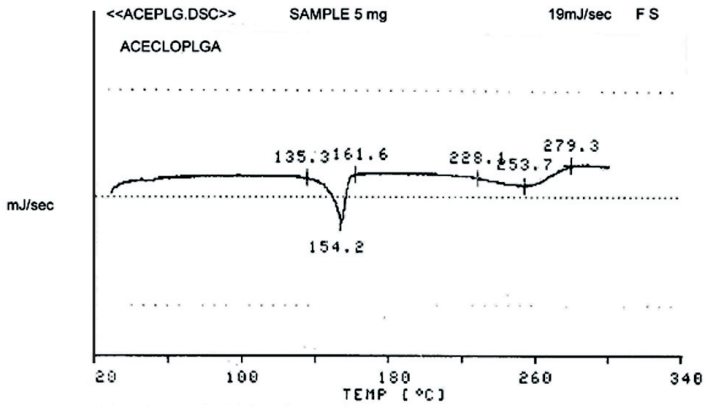

Figure 5. DSC of Aceclofenac Aceclofenac-PLGA Microsphere.

Stability Study

Microspheres were tested for physical and chemical behavior after stability testing. No prominent changes in the physical appearance of the microsphere were observed. The drug content at 40°C was reduced to 94.82% after 6 weeks. Good stability was observed at low temperature.

Table 5 and Figure 6 show the stability data and stability profiles from aceclofenac-PLGA microsphere.

Table 5. Stability Data for Aceclofenac-PLGA Microsphere.

Serial No.	Sampling interval	Drug content of microsphere			Physical appearance		
		5°C	25°C/ 60% RH	40°C/ 75% RH	5°C	25°C/ 60% RH	40°C/ 75% RH
0	0th day	100%	100%	100%			
1	7th day	100%	100%	99.45%	*	*	*
2	14th day	100%	99.51%	98.72%	*	*	*
3	21st day	100%	98.21%	97.65%	*	*	*
4	28th day	100%	97.91%	96.23%	*	*	*
5	35th day	100%	97.24%	95.43%	*	*	*
6	42nd day	100%	96.50%	94.82%	*	*	*

* No change

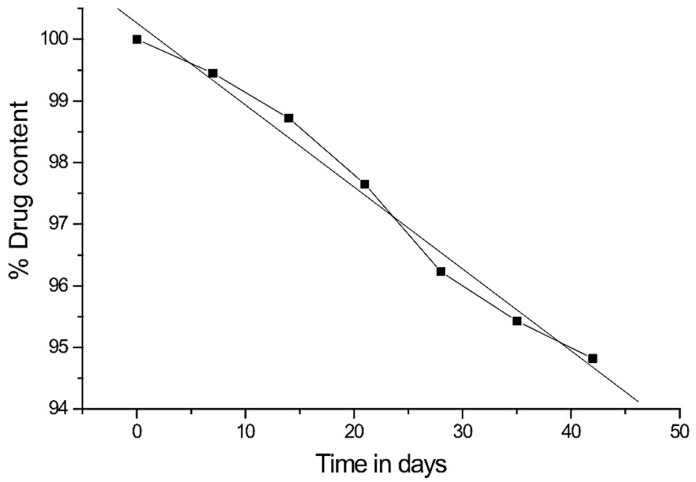

Figure 6. Stability Profile for Aceclofenac-PLGA Microsphere at 40°C.

CONCLUSION

Over the years, attempts have been made to control the time course and specificity of drug in the body through a variety of drug modifications and dosage forms. The need of making any drug microspheres is to produce a drug delivery system which is safe and capable of producing consistent therapeutic blood levels of drug in the body for required period of time. It also improves keeping and handling properties of the drug.

In the present investigations, attempts have been made, based on reported side effects and pharmacokinetic data, to prepare and evaluate the microsphere of Aceclofenac using PLGA polymer.

Aceclofenac is a NSAID. The PLGA microspheres were spherical with smooth surface. The percent yield was 78 %. The entrapment efficiency of the microsphere was 25%. The release of aceclofenac was satisfactory from microsphere. The release was retarded for around 9-10 hrs. In-vivo study indicated the Cmax at 5 hr. The complete elimination occurred in 48 hrs.

The microspheres were stable and no degradation of drug during preparation was observed.

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