Materials Science and Engineering C 72 (2017) 492–500

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Polymer based microspheres of aceclofenac as sustained release parenterals for prolonged anti-inflammatory effect

Manpreet Kaur, Sumit Sharma, VR Sinha *

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India.

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form 25 October 2016
Accepted 23 November 2016
Available online 25 November 2016

Keywords:
Microspheres
Aceclofenac
PLGA
PCL
Parenterals
Anti-inflammatory

ABSTRACT

Poly(lactic-*co*-glycolic acid) (PLGA) (75:25) and polycaprolactone (PCL) microspheres were fabricated for prolonged release of aceclofenac by parenteral administration. Microspheres encapsulating aceclofenac were designed to release the drug at controlled rate for around one month. Biodegradable microspheres were prepared by solvent emulsification evaporation method in different polymer:drug ratios (1:1, 2:1 and 3:1). After drug loading, PLGA and PCL microspheres showed a controlled size distribution with an average size of 11.75 μm and 3.81 μm respectively and entrapment efficiency in the range of 90 ± 0.72% to 91.06 ± 4.01% with PLGA and 83.01 ± 2.13% to 90.4 ± 2.11% with PCL. Scanning electron microscopy has confirmed good spherical structures of microspheres. The percent yield of biodegradable polymeric microspheres ranged between 30.95 ± 10.14% to 92.84 ± 3.15% and 47.33 ± 4.72% to 80 ± 3.60% for PLGA and PCL microspheres respectively. PLGA microspheres followed Higuchi release pattern while Korsmeyer-Peppas explained the release pattern of PCL microspheres. Stability studies of microspheres were also carried out by storing the preparations at 2-8 °C for 30, 60 and 90 days and evaluating them for entrapment efficiency, residual drug content and polymer drug compatability. *In-vivo* studies showed significant anti-inflammatory activity of microspheres upto 48 hours using the carrageenan induced rat paw oedema model.

1. Introduction

The poly(D,L-lactic-*co*-glycolic acid) (PLGA) and polycaprolactone (PCL) micro- and nano particulate systems for delivery of bioactive molecules, tissue engineering are widely studied. The use of PLGA and PCL polymeric microspheres for parenteral administration have gained appreciable attention over past few years and proved to be potential alternative therapeutic approach [1–4]. Moreover these polymers are well liked carrier materials for parenteral administration of certain drugs and/or unstable bioactive molecules as their control release mechanism will be useful in chronic diseases. This interest is due to numerous benefits offered by these systems which include established biocompatibility and biodegradability, ease of application, localized delivery for site specific action [5,6] sustained release, decreased drug dose with significant reduction in undesirable side effects and improved patient compliance. The reason for wide applicability of microspheres is due to their ability to encapsulate variety of drugs like small molecules, proteins, nucleic acids, biocompatibility, high bioavailability and can produce sustain release for longer periods of time [7,8].

Treatment options for osteoarthritis include symptomatic relief with simple analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), intraarticular [9] injected glucocorticoids and hyaluronic acid preparations. Non-pharmacological measures range from physical exercise and weight loss to joint lavage and eventually surgical joint replacement [10]. Since the introduction of acetylsalicylic acid in nineteenth century, NSAIDs have become widely acceptable as analgesics and anti-inflammatory drugs for treatment of such conditions. NSAIDs act by inhibiting cyclooxygenase (COX), a key enzyme in inflammation cascade. They have varying degree of specificity for two isoforms of COX. Aceclofenac is a non-selective COX inhibitor. Aceclofenac shows stimulatory effects on glycosaminoglycan in human osteoarthritic cartilage and chondroprotective effects, mediated by suppression of metalloprotease production and proteoglycan release in rabbit articular chondrocytes and human rheumatoid synovial cells [11]. Studies performed on human blood assays show that aceclofenac and its metabolite 4′-hydroxyaceclofenac inhibit COX-2 (IC50 = 0.77 and 36 μM, respectively) but have relatively less effect on COX-1 (IC50 ≥ 100 μM) [12]. Aceclofenac is a derivative of diclofenac which has lesser side effects related to gastrointestinal complications as compare to diclofenac. Clinical trials reported that aceclofenac is better tolerated and favored by patients orally as well as parenterally in inflammation as compare to diclofenac [13,14].

Present research aims to formulate sustained release microspheres of aceclofenac for parenteral administration, using two different polymers *i.e.* PLGA and PCL. Due to short half life ($t_{1/2}$ = 3–4 h) of aceclofenac, frequent dosing is required [9,15]. As arthritis affects people mainly above 60 years of age, which are not very comfortable with

* Corresponding author.
E-mail address: sinha_vr@rediffmail.com (V.R. Sinha).

http://dx.doi.org/10.1016/j.msec.2016.11.092
0928-4931/

frequent dosing, hence a need of prolonged release formulation is foreseen. Moreover the side effects associated with fluctuations in drug plasma concentration paved the way for the development of long acting formulation of aceclofenac.

2. Experimental methods

2.1. Materials

Aceclofenac was procured *ex-gratis* from Cadila Pharmaceuticals, Ahmedabad, India. Polymers (PLGA and PCL) were supplied by Union Carbide, India. Solvents including acetonitrile and methanol of HPLC grade (Fisher Scientific, Mumbai, India) were used for HPLC analysis. Chemicals including di-sodium hydrogen phosphate, potassium dihydrogen phosphate, polyvinyl alcohol and dichloromethane were supplied by Central Drug House (P) Ltd., New Delhi, India. Nylon filters, pore size 0.22 μ and 0.45 μ (Millipore, USA) were used for filtration of solvents and prepared samples for HPLC analysis.

2.2. Preparation of aceclofenac loaded polymeric microspheres

Solvent emulsification-evaporation method was used for preparation of microspheres [16,17]. Different batches of varying polymer: drug ratios (1:1, 2:1, 3:1) were prepared using two polymers, PLGA (75:25) and PCL.

2.2.1. Preparation of PLGA (75:25) microspheres

Accurately weighed PLGA (150, 300 and 450 mg) was dissolved in 9 ml of dichloromethane (DCM) and aceclofenac (150 mg) was dissolved in 1 ml methanol. Both the solutions were mixed together. The mixture was added at once to 100 ml of polyvinyl alcohol (PVA) solution (0.5%) under constant homogenization at 14,000 rpm. Three homogenization cycles each of 10 min were given with a rest period of 10 min, resulting in formation of an emulsion. This emulsion was stirred on magnetic stirrer for 10–12 h in order to remove the solvent at room temperature. The rigidized microspheres were washed thoroughly with water to remove the adhered PVA from the surface as well as loosely adsorbed drug on the surface. Finally, the microspheres were collected by filtration using vacuum pump followed by air drying. The three prepared batches were named as PLGA 11, PLGA 21 and PLGA 31.

2.2.2. Preparation of PCL microspheres

Precisely weighed PCL (150, 300 and 450 mg) and drug (150 mg) were dissolved in 10 ml of dichloromethane (DCM). Further same procedure was followed as for the preparation of PLGA microspheres. The three prepared batches were named as PCL 11, PCL 21 and PCL 31.

2.3. Characterization of microspheres

Microspheres were suspended in 0.1% tween 80, homogenized at 10,000 rpm followed by sonication for 60 s. Mean particle size and particle size distributions were analyzed by laser diffractometry using Malvern Mastersizer 2000. In order to investigate surface morphology of the prepared dried microspheres, these were mounted onto metal stubs using double-sided adhesive tape. Subsequently, the stubs were vacuum coated with gold using fine coat ion sputter (JFC 1100, JEOL Japan) under reduced pressure to render them electrically conductive. Microspheres were then examined with scanning electron microscope (SEM) (JEOL JSM 6100, Tokyo, Japan). The accelerating voltage was kept constant initially at 15.0 kV then further reduced to 10.0 kV under argon atmosphere.

2.4. Determination of entrapment efficiency and yield

All prepared batches of microspheres (10 mg) were dissolved in 10 ml of DCM to obtain the stock solution. The solution was vortexed using cyclo mixer. The stock solution was suitably diluted with DCM to prepare the samples to be analyzed, spectrophotometrically at 277.5 nm. The percent yield and percent entrapment efficiency was calculated using the Eqs. (1) and (2), respectively.

$$\%yield = \frac{Weight\ of\ microspheres\ obtained}{Total\ weight\ of\ aceclofenac\ and\ polymer} \times 100 \quad (1)$$

$$\%\text{entrapment efficiency} = \frac{\text{Weight of incorporated drug}}{\text{Weight of aceclofenac used in formulation}} \times 100 \quad (2)$$

2.5. Differential scanning calorimetric (DSC) studies

DSC studies of drug, polymer and microspheres were carried out to define physical state of drug in these carriers. It also explains the possibility of any interaction between the drug and polymer(s) within the network of polymeric matrix in microspheres. Small amount of samples were placed in hermetically sealed aluminium pans and heated from 30 °C to 300 °C at a heat flow rate of 10 °C min^{-1} under nitrogen spurge of 50 cm^3 min^{-1} (Mettler Toledo STAR system, Switzerland). The melting points of samples were recorded as endotherms.

2.6. Fourier transform infrared (FTIR) spectroscopy studies

FTIR spectroscopy was performed for the prepared polymeric microspheres by Spectrum Two Perkin Elmer FT-IR, USA, using potassium bromide pellets in the scanning region of 4500 to 500 cm^{-1}. The obtained spectra of pure aceclofenac, polymers and aceclofenac loaded microspheres were compared to identify molecular structure relationship between drug and polymer(s).

Table 1
Percent yield and entrapment efficiency data of microspheres prepared with PCL and PLGA (75:25) (n = 3).

Batch code	Batch specification	Particle size (μm)	% Yield ± S.D.	% EE ± S.D.
PCL 11	PCL (P/D ratio 1:1)	2.705	47.33 ± 4.72	83.01 ± 2.13
PCL 21	PCL (P/D ratio 2:1)	3.574	62.66 ± 7.57	89.98 ± 2.27
PCL 31	PCL (P/D ratio 3:1)	5.136	80 ± 3.60	90.4 ± 2.11
PLGA 11	PLGA (P/D ratio 1:1)	2.560	30.95 ± 10.14	90 ± 0.72
PLGA 21	PLGA (P/D ratio 2:1)	3.533	57.51 ± 3.23	90.48 ± 5.90
PLGA 31	PLGA (P/D ratio 3:1)	5.260	92.84 ± 3.15	91.06 ± 4.01