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Studies of *in vitro* Evaluation and Formulation of Aceclofenac Loaded PLGA Microspheres

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Abstract: The aim of this research was to study the influence of formulation parameters in the preparation of sustained release aceclofenac loaded PLGA microspheres by emulsion solvent diffusion technique. The methods used in this components and their concentration necessary for organogels formation were evaluated using phase diagram Solubility of aceclofenac was determined, Characterization of Poly (DL-lactide)-co-glycolide (PLGA) polymer, solubility assessment of aceclofenac, drug-excipients compatibility studies, *in vitro* analytical method development , preparation of aceclofenac-loaded PLGA microspheres, characterization of the formulations. Prepared microspheres were optimized and evaluated for different parameters and best formulation was subjected to *in vitro* drug release studies. The prepared microspheres were white, free-flowing and almost spherical in shape. *In vitro* drug release studies were carried out up to 24 h in three different pH media, i.e., 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8) and phosphate buffer (pH 7.4). The drug-polymer concentration of dispersed phase influences the particle size and drug release properties. In nut shell it may be concluded that sustained release aceclofenac microspheres can be successfully prepared and used parenterally with increased therapeutic value and reduced side effects.

Key words: Tween80, solvent evaporation, toxicity, biodegradable, excipients

INTRODUCTION

Aceclofenac, 2-[(2,6-dichlorophenyl) amino] phenylacetoxyacetic acid, a novel anti-inflammatory drug of choice in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Researchers have attempted development of oral drug delivery systems for aceclofenac (Yong *et al*., 2005). The chronic oral administration of aceclofenac tends to cause severe gastric irritation (Tessari *et al*., 1995). Topical administration of aceclofenac offers the advantage of enhanced drug delivery to the affected areas, by passing gastric irritation. The clinically efficiency have evaluated of topical aceclofenac cream (1.5% w/w) in patients (Yang *et al*., 2002). The formulation showed improved therapeutic efficacy. In the formulated micro-emulsion containing aceclofenac (3%w/w) for topical delivery (Yamazaki *et al*., 1997).

This action is supposed to mediate via its intracellular conversion to active metabolites namely diclofenac, the cyclooxygenase 1 and 2 inhibitor and 4-hydroxydiclofenac, the cyclooxygenase-2 inhibitor (Akimoto *et al*., 2000). It also stimulates glycosaminoglycan synthesis in human osteoarthritic cartilage by inhibition of interleukin-1beta (IL-1β) and suppresses cartilage degeneration by inhibiting IL-1β mediated promatrix metalloproteinase production and proteoglycan release (Gowda *et al*., 2006). Aceclofenac is especially well-tolerated among the non-steroidal anti-inflammatory drugs and have lower incidence of gastrointestinal (gi) adverse effects (Raber *et al*., 2007). It also have lower incidence of gi bleeding, abdominal pain and arterial hypertension than meloxicam and a lower incidence of gi bleeding, abdominal pain, liver toxicity, thromboembolic cardiovascular events, arterial hypertension and edema than rofecoxib (Brogden and Wiseman, 1996).

Aceclofenac is indicated and widely used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and scapulohumeral periarthritis (El-Kousy, 1999; Najib *et al*., 2004). The usual dose of aceclofenac is 100 mg twice daily (Kim *et al*., 2001) and it is absorbed rapidly when taken orally and its analgesic effects begin within 30 min of administration

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(Dashora *et al*., 2006). Following oral dose, it reaches a peak plasma concentration within 1-3 h (Patil and Kasture, 2007). Since, aceclofenac is indicated for various pains and inflammatory conditions, its accelerated onset of action and sustained release along with decreased incidence of dose related toxicity is desirable. Keeping in mind the above requirements we prepared aceclofenac loaded Poly (DL-lactide)-co-glycolide (PLGA) microspheres, characterized and evaluated for drug release pattern. PLGA is biodegradable and biocompatible polymer and widely used in controlled release drug delivery systems. Usual therapeutic dose is 100 mg twice daily and half life is 3-4 h; thus it is necessary to be administered frequently in order to maintain the desired concentration. Therefore, aceclofenac is an ideal candidate for sustained release formulation, resulting in more reproducible drug absorption and reducing the risk of local irritations compared to single dosage forms.

The solvent evaporation method is popularly used for microsphere preparation because of its simplicity, reproducibility and fast processing with minimum controllable process variables that can be easily implemented at the industrial level (Kim *et al*., 2002; Dashora *et al*., 2006). But it is frequently used for water-insoluble drugs, as the entrapment efficiency of water-soluble drugs is low due to drug loss from the organic emulsified polymeric phase before solidification of polymer in the microspheres (Niwa *et al*., 1993, 1994).

The objective of this study was to encapsulate aceclofenac with biodegradable.

Polymer rosin, the effect of different formulation variables such as concentration of drug, Polymer, polyvinyl alcohol and solvent, the effect of these variables on particle size distribution, encapsulation efficiency and its *in vitro* release behavior. The drug was targeted to the colon and their aligned area for their local effect.

MATERIALS AND METHODS

Chemicals: Aceclofenac and Poly (DL-lactide)-co-glycolide (PLGA) were provided (2009) by Venus Medicine Research Center (VMRC), Baddi, H.P., India. Acetonitrile was HPLC grade and all other chemicals used were of analytical grade.

Characterization of Poly (DL-lactide)-co-glycolide (PLGA) polymer PLGA polymer was characterized by gel permeation chromatography for its molecular weight and molecular weight distribution with mixed bed column (SDV 10 μ, 8X300 μ, PSS polymer standard service GmbH) using chloroform (Injection volume: 100 μL) as mobile phase at 1 mL min^{-1} flow rate for 15 min and 30°C using Refractive index detector and GPC for Class-Vp software as analyser.

Solubility assessment of aceclofenac: The solubility of the drug was assessed in Ethyl acetate, Dichloromethane and Benzyl alcohol by serial addition of the known amount of drug into these solvents and observing visually for complete solubilisation.

Drug-excipients compatibility studies: Excipients are integral components of almost all pharmaceutical dosage forms thus it is mandatory to detect any possible physical or chemical interaction of the drug with the excipients since the excipient can affect the bioavailability and stability of the drug. The drug and the excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, easy to administer and safe. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies have a considerable importance.

***In vitro* analytical method development:** Different concentrations of aceclofenac were prepared from drug stock solution (50 μg mL^{-1}) prepared in phosphate buffer pH 7.4 and analytical parameters were determined using Reverse Phase High-performance liquid chromatography (RP-HPLC, Waters) system consisting of a photodiode array detector. The column was a reverse phase (Inertsil ODS C18, 250×4.6 mm, 5 μm). The mobile phase was acetonitrile/10 mM phosphate buffer (pH 7.0) (40:60, v/v) and the flow rate was 1 mL/1 min. The temperature of the system was maintained at room temperature and the detection wavelength was 274 nm.

Preparation of Aceclofenac-loaded PLGA Microspheres PLGA was first dissolved in ethyl acetate (2 mL) and aceclofenac was further dissolved in polymer solution. This polymer-drug solution was then added to Poly Vinyl Acetate (PVA) surfactant solution under continuous stirring at different speeds to obtain a primary emulsion in aqueous phase. Ethyl acetate was then allowed to evaporate. The solid microspheres were washed thoroughly using distilled water, passed through sieve (BSS # 120), freeze dried and evaluated.

Characterization of the formulations

Drug content in microspheres: Microspheres (5 mg) were dissolved in 5 mL of Dichloromethane and then evaporated under stream of nitrogen. To this 10 mL acetonitrile was added. One milliliter of this solution was then diluted to 3 mL and filtered through 0.22 μ filter paper. The amount of drug in the test sample was determined by RP-HPLC method.

The drug loading capacity and encapsulation efficiency of the microspheres were calculated according to the following equations:

$$\text{Loading capacity} = \frac{\text{Total drug in microsphere}}{\text{Total weight of microsphere}}$$

$$\text{Entrapment Ef ficiency} = \frac{\text{Total drug in microsphere}}{\text{Total weight of microsphere}}$$

Particle size measurements: Particle sizes of microspheres were determined using scanning optical microscopy technique.

Drug content analysis: Ten milligram accurately weighed portion of microspheres were taken in a clean 100 mL volumetric flask and dissolved in about 2 mL of acetone and the volume was made up to the mark with buffer pH 7.4. After filtration and dilution, samples were analyzed spectrophotometrically and the amounts of drug encapsulated in the microspheres were calculated. The drug content of each sample was determined in triplicate and the results were averaged. The entrapment efficiency of microspheres was calculated by dividing the actual drug content to the theoretical drug content of microspheres.

Redispersability: Prepared sodium carboxymethyl cellulose (0.5% w/v) in buffer pH 7.0. To 20 mL of sodium CMC solution SPAN 80 solution (10 mL, 1% in buffer pH 7.0) and NaCl (170 mg) was added and volume was made 100 mL in buffer pH 7.4. 300 mg of microspheres were taken and to this 1 mL of dispensing fluid was added. The microspheres were hand shaken and a small amount of sample was taken on a slide and observed under microscope.

Injectability: Microspheres were redispersed in the dispensing fluid (30% w/v) and passed through 22 G needle to determine the injectability behaviour of the microspheres.

***In vitro* drug release studies:** *In vitro* release of STP from microspheres was evaluated in phosphate buffer (pH 7.4). Amount of microspheres equivalent to 20 mg of STP were transferred to the prewarmed dissolution media (20 mL) and maintained at 37±0.5°C under stirring at 50 rpm. Samples were withdrawn every hour up to 6 hours and the volume was replaced immediately by fresh phosphate buffer. The sample withdrawn was centrifuged (3000 rpm, 15 min). The supernatant solution was filtered and analyzed for STP content by measuring absorbance in a UV-spectrophotometer (Shimadzu UV-1700, Pharmaspec) at 229.5 nm by the first-derivative spectrophotometric method (Saudagar *et al*., 2007) using phosphate buffer (pH 7.4) as blank. Results were expressed as mean±SD of 3 experiments. Statistical models are extensively used in diversified areas to strengthen the art of drug formulation (Dubey and Parikh, 2004; Govender *et al*., 2005).

RESULTS

Characterization of poly (DL-lactide)-co-glycolide (PLGA) polymer: Unimodal and symmetrical GPC trace was obtained (Fig. 1) thus confirming the purity of the polymer. The polymer PLGA was having weight average molecular weight of 130 kDa, number average molecular of 76 kDa and polydispersity of 1.7 which was sufficient enough for current application. Optimization of drug: Polymer concentration, PVA concentration and speed are shown in Table 1. Solubility studies of aceclofenac revealed soloubility of aceclofenac in following order Benzyl alcohol>Ethyl acetate> Dichloromethane and are shown in Table 2. *In vitro* analytical method development by validation parameters for calibration over a range of 1 to 50 mg mL^{-1} is summarized. The rapid diffusion of acetone into the aqueous phase causes a remarkable decrease in interfacial tension between the organic and aqueous phase and hence finer microspheres are obtained (Niwa *et al*., 1994).

At least one stabilizer is necessary for microsphere formation and suspension stabilization. Tween 20, Tween 40 and Tween 80 were used as surfactants to prepare blank microspheres; however, using Tween 20 and Tween 40, microsphere formation was not achieved successfully independent of their concentration. Microspheres with superior topographical characteristics were obtained when Tween 80 was used as the emulsifier (Fig. 2). This might be due to better emulsification capability of Tween 80 as compared with Tween 20 or Tween 40 (Kibbe, 2000) The pH of Tween 80 aqueous solution is in the range of 6.0 to 6.5 and hence did not warrant pH adjustment

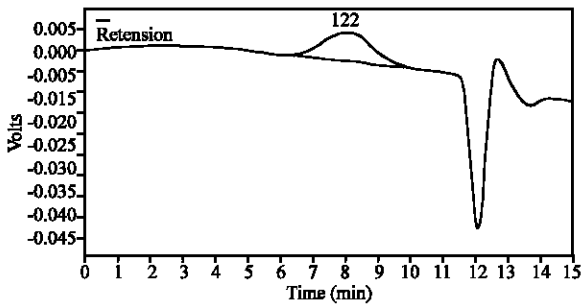

Fig. 1: Representative gel permeation chromatogram of PLGA (75: 25)

Table 1: Optimization of drug: polymer concentration, PVA concentration and speed

Formulation code	Drug (mg)	Polymer (mg)	Ethyl-acetate (mL)	PVA (%)	Speed (rpm)	Particle size (average diameter, μm)	Mean loading capacity (%)	Mean entrapment efficiency (%)
V 1	40	40	2	0.5	500	28.22	22.08	55.12
V 2	40	80		0.5	500	53.28	24.64	60.23
V 3	40	120		0.5	500	98.56	25.26	63.17
V 4	80	40		0.5	500	38.24	26.38	66.11
V 5	80	80		0.5	500	45.24	27.48	67.89
V 6	80	120		0.5	500	78.67	32.42	79.72
V 7	120	40		0.5	500	36.26	27.11	67.11
V 8	120	80		0.5	500	98.23	29.18	73.18
V 9	120	120		0.5	500	109.10	32.49	79.85
V 10	80	120		0.2	500	104.23	34.04	-
V 11	80	120		0.5	500	78.67	32.42	-
V 12	80	120		0.8	500	69.86	31.94	-
V 13	80	120		1.0	500	60.93	31.26	-
V 14	80	120		1.0	500	60.86	31.14	-
V 15	80	120		1.0	1000	43.65	30.28	-
V 16	80	120		1.0	1500	32.15	27.34	-

Table 2: Solubility of aceclofenac in different solvents

Solvents	Solubility (mg mL^{-1})
Dichloromethane	<50
Ethyl acetate	<130
Benzyl alcohol	<150

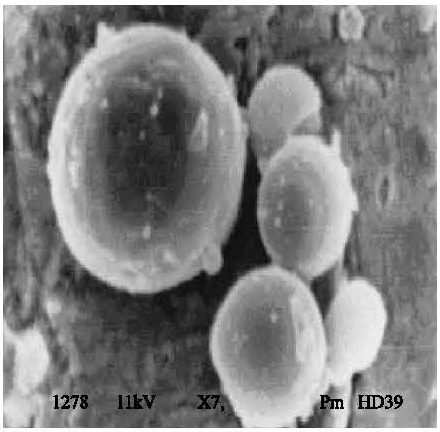

Fig. 2: Scanning electron micrograph (SEM) of cellulose acetate microspheres

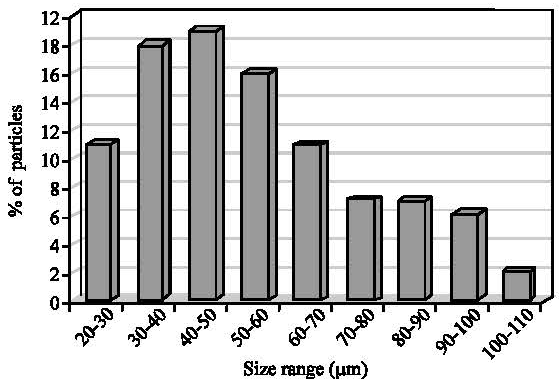

Fig. 3: Particle size distribution of drug loaded microspheres

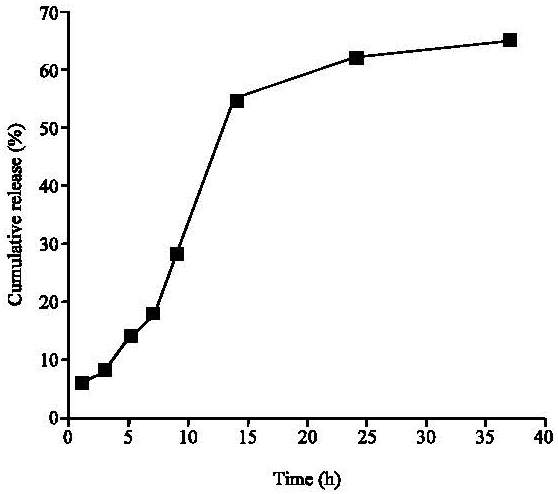

Fig. 4: *In vitro* release of acelofenac from microsphere

(Salamone and Wodzinski, 1997). *In vitro* studies showed in this batch of Aceclofenac Loaded PLGA Microsphere showed cumulative percentage release of 10 to 65% respectively for 40 h (Fig. 4).

Evaluation of formulation variables: As mentioned previously, the effect of different formulation variables on microspheres properties including entrapment efficiency and particle size distribution were evaluated. Variables studied in this investigation were as follows. Drug polymer ratio, volume of dichloromethane and polyvinyl alcohol concentration in the external phase of the emulsion.

Particle size measurements: All the particles of the selected batch V15 were spherical in shape (Fig. 2) and the particle size was in the range of 20- 110 μm (Fig. 3), of which 53% particles were in the range of 30-60 μm and

75% of the particles were in the range of 20-70 μm which is very much suitable for intramuscular parenteral drug delivery system.

Redispersability: The microspheres were found to be uniformly dispersed in the dispensing fluid. Further they were present as single particles when observed under microscope. It was also observed that particles were suspended in the fluid for more than 3 min which is very much sufficient for injection.

Injectability: The suspension of the microsphere was easily passed through 22 G needle therefore no problem will arise due to injectability properties of the prepared formulations.

DISCUSSION

This research study indicates that aceclofenac loaded PLGA microspheres can be successfully formulated for parenteral drug delivery of the drug. All the preparation having ratio 1:1 showed good release properties. As the amount of polymer increased, drug release was decreased. This is because smaller the particle size, larger the surface area available for drug release. This may be attributed to the film forming ability of the lipospheres pellet which reduces transepidermal water loss similar to solid lipid nanoparticles (Mei *et al*., 2003). The major finding of this study can be concluded as aceclofenac loaded PLGA microspheres with mean size of about 54 μm can be prepared using solvent-evaporation technique with PVA as stabilizer and ethyl acetate as solvent. Microspheres belong to the above category and are utilized for controlled drug delivery. Microspheres were prepared by the emulsion solvent evaporation method and evaluated. Aceclofenac is a novel anti-inflammatory drug with multidisciplinary therapeutic activity in pain and inflammatory conditions. The loading capacity increases with increase in initial drug polymer ratio. The formulated microspheres can be used for IM delivery of the drug with sustained effect. Dose of the drug is reduced with consequently decrease in dose related toxicity of the drug.

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