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Diclofenac sodium incorporated PLGA (50:50) microspheres: formulation considerations and in vitro/in vivo evaluation

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Abstract

Recently, considerable interest has been focused on the use of biodegradable polymers for specialized applications such as controlled release of drug formulations; meanwhile, microsphere drug-delivery systems using various kinds of biodegradable polymers have been studied extensively during the past two decades. Poly (lactide-co-glycolide) (PLGA) polymers have been proven to be excellent drug carriers for microparticulate systems due to their advantages, e.g. biocompatibility and regulatory approval. The administration of nonsteroidal anti-inflammatory drugs (NSAIDs) into the intra-articular cavity in patients with chronic inflammatory disease is complicated due to the short duration of effect. In the present study, controlled-release parenteral formulations of diclofenac sodium (DS), a commonly used NSAID, were prepared for intra-articular administration, and evaluated in vitro for particle size, yield, drug loading, surface morphology and release characteristics. For in vivo studies, Technetium-99m labelled polyclonal human immunogammaglobulin (^{99m} Tc-HIG) was used as the radiopharmaceutical to demonstrate arthritic lesions by gamma scintigraphy. Evaluation of arthritic lesions post-therapy in rabbits showed no significant difference in the group treated with PLGA (50:50) (mw 34 000) DS microspheres compared to control groups.

Keywords: Poly (lactide-co-glycolic acid) microspheres; Diclofenac sodium; Solvent-evaporation method; Experimental arthritis; Scintigraphic imaging

1. Introduction

Diclofenac sodium (DS), is a sodium salt of an aminophenyl acetic acid and is commonly utilized in the treatment of arthritic disorders, such as ankylosing spondylitis, rheumatoid arthritis and osteoartritis. As with nonsteroidal anti-inflammatory drugs (NSAIDs), side effects result in gastrointestinal mucosal damage, irritation and bleeding. Parenteral polymeric dosage forms providing sustained release of DS, cause a reduction in the localized gastrointestinal disturbance occuring as a result of frequent oral administration of the drug.

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Poly (lactide-co-glycolic acid) (PLGA), a copolymer of poly(lactic acid) and poly(glycolic acid), has been studied extensively as a polymeric carrier for biodegradable microspheres. Thus, mainly due to their long history of safe human use in the form of surgical sutures and their commercial availability in various monomer ratios and molecular weights, a wide variety of drugs ranging from small molecular weight therapeutic agents to peptide hormones, antibiotics and chemotherapeutic drugs have been studied using these biodegradable polymers. PLGA microparticles have proved to be successful drug-delivery systems for incorporating different classes of drugs, such as NSAIDs, peptides like LHRH agonists and steroid hormones. By selection of the appropriate polymer composition with a known rate of degradation, the polymers can be exploited to produce a drug-delivery system which releases an active agent at a predetermined rate.

Several methods have been used in preparation of microspheres, both for natural and synthetic polymers. Emulsification by solvent evaporation is the most popular method for preparing PLGA microspheres, due to its reproducibility and uniformity of particle size.

2. Materials and methods

Diclofenac sodium (active substance), organic solvents and other chemicals used for microsphere preparation, analytic procedures and in vivo studies were purchased from commercial suppliers and used without further purification. Diclofenac sodium (Novartis, Switzerland), PLGA (50:50) (34 000 and 88 000 Da) was from Medisorb, USA. Sodium oleate (SO), methanol, and polyvinyl alcohol (PVA) were purchased from Merck, Germany. Methylene chloride was supplied by Quimon, Spain. Polyclonal human immunoglobulin kits were obtained from Mallinckrodt, Holland. Tc-99m as pertechnetate was obtained from a generator (Amersham, England).

2.1. Preparation of microspheres

DS microspheres were prepared by the emulsification/solvent evaporation process (Fig. 1). In order to form an o/w-type emulsion, 30 mg of the drug were dissolved in methanol and added to the polymer solution (300 mg PLGA 50:50) in methylene chloride. Then this dispersion was emulsified into the aqueous continuous phase (100

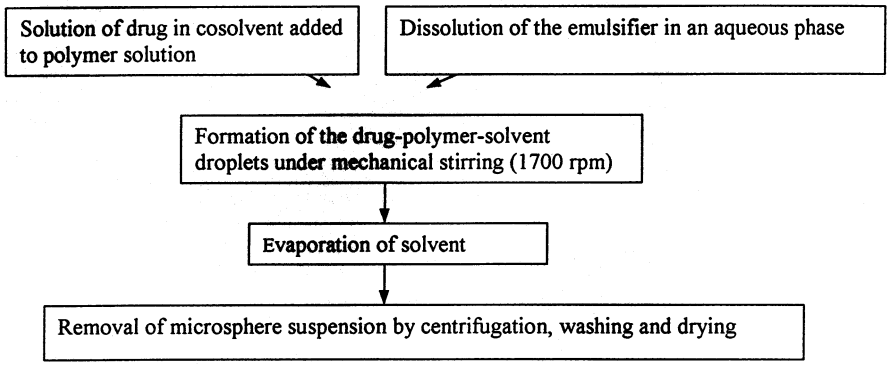

Fig. 1. Flow diagram of the preparation of DS-incorporated PLGA (50:50) microspheres by the solvent-evaporation process.

Table 1
Characterization of DS-containing PLGA (50:50) microspheres

Code	Yield value (%)	Particle size (%) 50 (μm)	Total drug content (%)
A	70	9.39	12.7
B	68	5.77	16.1

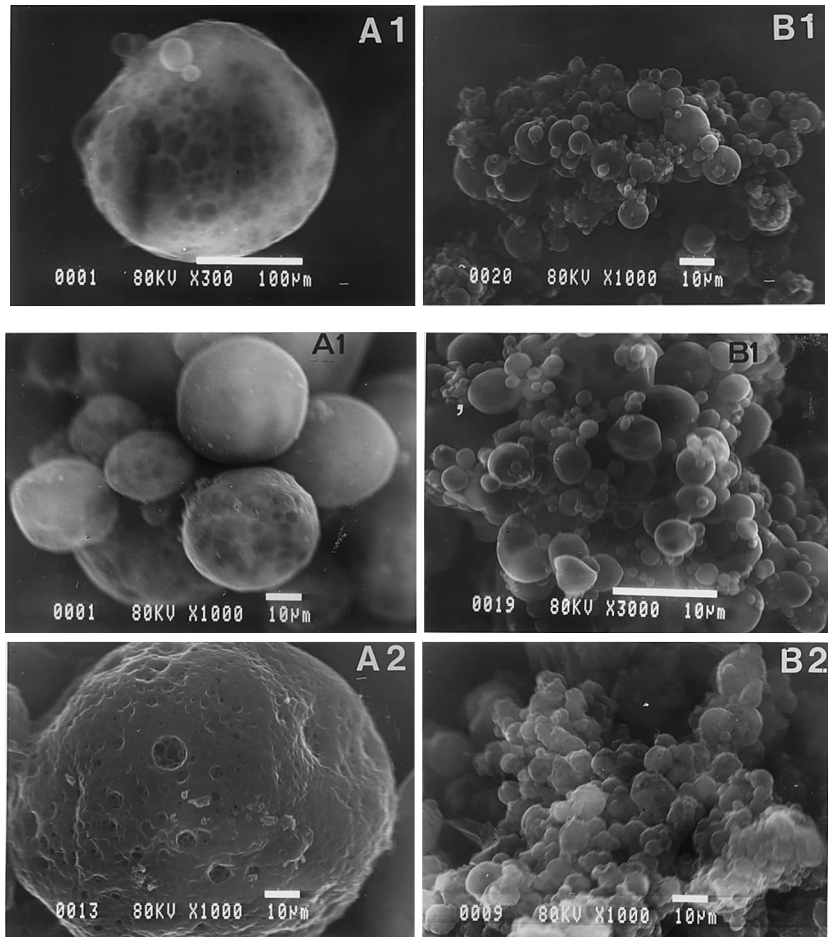

Fig. 2. Surface morphology of DS-containing PLGA microspheres. Scanning electron microscopy (SEM) photograph: (A1) molecular weight of 34 000 Da (before in vitro release); (A2) molecular weight of 34 000 Da (after in vitro release); (B1) molecular weight of 88 000 Da (before in vitro release); (B2) molecular weight of 88 000 Da (after in vitro release).

ml), consisting of 500 mg PVA:SO (4:1) as stabilizer. The medium was stirred vigorously at 1700 rpm for 2 h to allow the complete evaporation of methylene chloride. The resulting microspheres were collected by centrifugation, washed with water, and dried at room temperature.

Blank microspheres were prepared in a similar way to be used as a control in the characterization studies. Several batches of DS and blank microspheres were prepared. The respective batches were mixed together for physicochemical characterization.

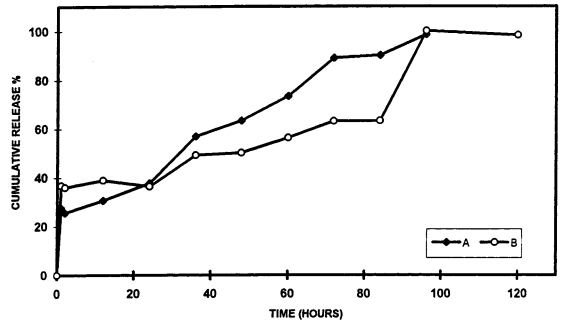

Fig. 3. In vitro release profiles of DS microspheres.

2.2. Characterization of the microspheres

2.2.1. Drug content

The dried PLGA (50:50) microspheres (50 mg) were kept in ultrasonic bath for 15 min after the addition of 10 ml pH 6.8 phosphate buffer and filtered through 0.22 μm Millipore filters. The absorbance of the filtered liquid was measured at 276 nm (Shimadzu UV/VIS spectrophotometer) in order to determine the amount of drug present on the surface of the microspheres. The polymer was dissolved by adding 5 ml of methylene chloride to the dried and weighed microspheres, and finally the polymer precipitated after the addition of methanol. The amount of entrapped drug was measured by an UV spectrophotometer.

2.2.2. Particle size analysis

A small amount of microspheres was suspended in an aqueous solution containing 0.1% Tween 80

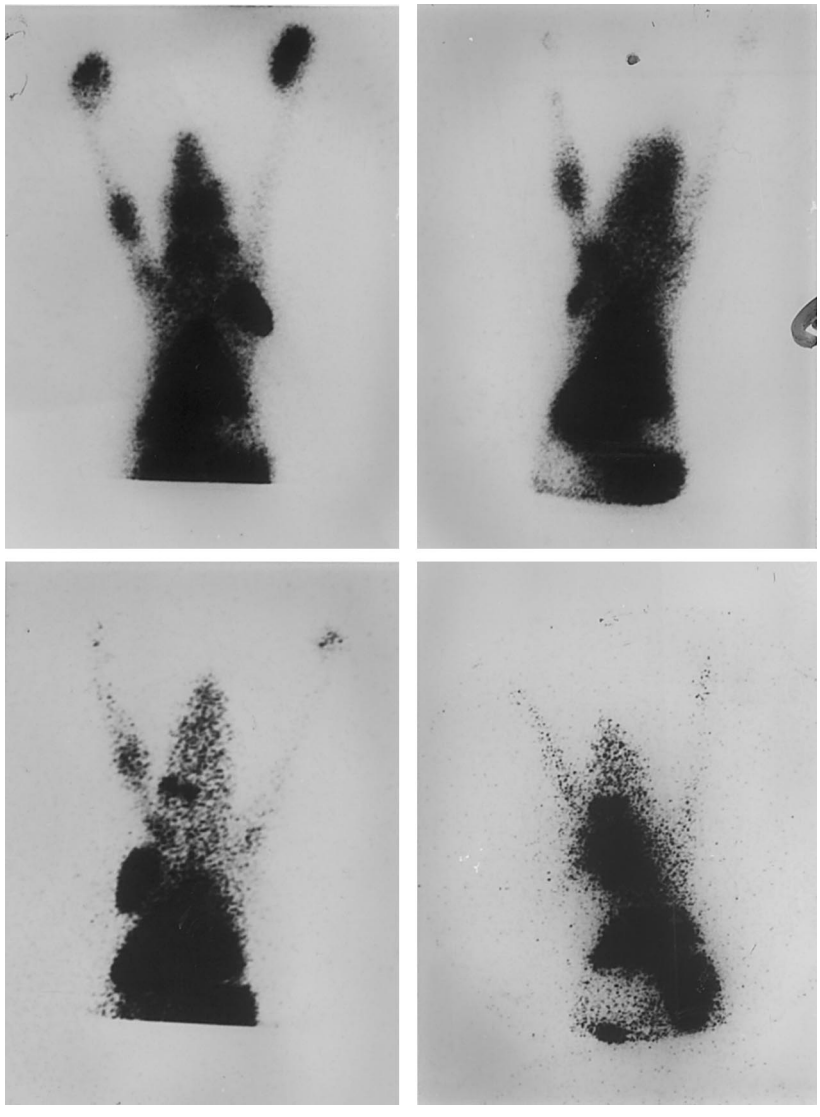

Fig. 4. Scintigrams of arthritic rabbits taken 4 h after the administration of ^{99m} Tc-HIG.

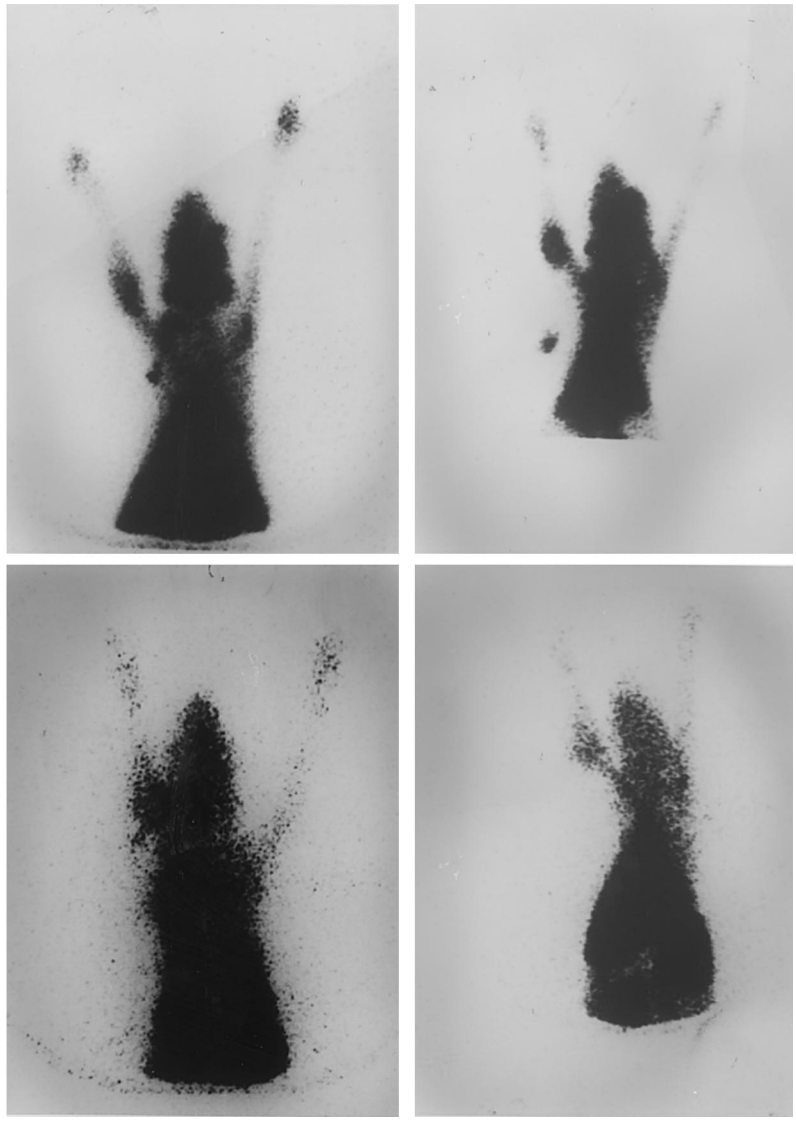

Fig. 4. (*Continued*)

and sonicated for 5 min before the particle size analysis was done by laser diffraction (Malvern Mastersizer).

2.2.3. Surface morphology of PLGA microspheres

The appearance of the microspheres was evaluated by scanning electron microscopy (Jeol-SEM ACID-10 Device at 80 KV). Microspheres were mounted on metal stubs with conductive silver paint and sputtered with a 150 Å thick layer of gold in a BIO-RAD apparatus.

2.2.4. In vitro release studies

Fifty milligrammes of microspheres were placed in 25 ml (pH 6.8) of phosphate-buffered saline (PBS) in a 50 ml glass vial. The microspheres in the medium were shaken at 50 cpm in a thermostated bath at 37°C. Samples were withdrawn at appropriate time intervals and filtered through a Whatman 45 filter and replaced by an equal volume of dissolution medium. Subsequently, they were assayed using an UV spectrophotometer.

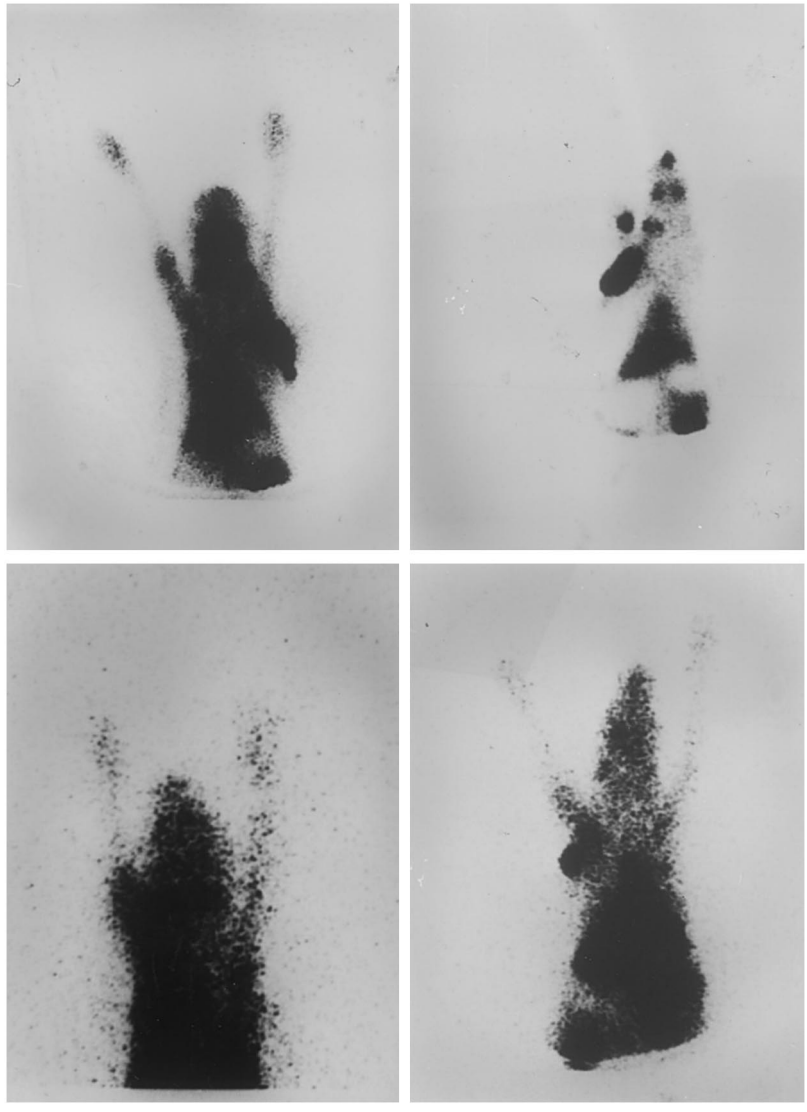

Fig. 4. (*Continued*)

2.3. In vivo studies

White female New Zealand rabbits ($n = 15$) weighing approximately 3 kg were used for in vivo studies. Mono-articular arthritis was induced in the left knee joints of rabbits by modification of the method applied by Ratcliffe et al. (1987). Ovalbumin, which was used as the antigen, was emulsified with Freund's Complete Adjuvant. A physiological solution (0.9% NaCl) of albumin at a concentration of 20 mg/ml was emulsified with an equal volume of Freund's Complete Adjuvant. In order to obtain homogenous dispersion, the emulsion was passed through a homogenizer. One milliliter of the emulsion sample was injected into the left knee joints of each rabbit. The contralateral knees were used as the control joints.

Technetium-99m labeled polyclonal human immunogammaglobulin (^{99m}Tc-HIG) was used as the radiopharmaceutical to demonstrate arthritic

lesions by gamma scintigraphy before therapy (Rubin et al., 1989; Nasirideen et al., 1998). Four days after the induction of arthritis, each rabbit was administered with 1 mCi (0.2 ml) of ^{99m}Tc-HIG intravenously through the ear vein in saline solution. Four hours after the administration of the radiopharmaceutical, static images were obtained from the posterior position with a gamma camera using a LEAP collimator. Three to five hundred kilocounts were collected to obtain an image. Regions of interest were drawn over both arthritic (target) and contralateral (nontarget) knee joints. The radioactive count ratios obtained from the equal regions of interest of arthritic (A) and control areas (C) were used to determine the A:C ratios.

The labeling efficiency of ^{99m}Tc labeled HIG was determined by impregnated thin-layer chromatography (ITLC), using ITLC-SG mini strips and saline solvent (Saha, 1992).

DS-containing microspheres were used as the test sample. Blank microspheres and DS solution in buffered saline were used as control samples. Samples were administered to rabbits intra-articularly 1 d after the demonstration of arthritic lesions. DS-containing preparations were administered at a dose of 2 mg/0.5 ml to each rabbit in their respective groups ($n = 5$). Blank microspheres and DS solution in buffered saline were administered as stated above (0.5 ml) to each rabbit in their respective groups ($n = 5$).

Evaluation of arthritis was performed by the i.v. injection of 1 mCi of ^{99m}Tc-HIG, as explained before on days 3, 17 and 30. Four hours after the administration of the radiopharmaceutical, scintigraphic images of rabbits were obtained as described previously (Tunçay et al., 2000). Regions of interest were created in the target and non-target areas, and the radioactivity count ratios (A:C) in each group were determined. The average values of each group, with their standard deviations are presented in Table 2.

3. Results and discussion

PLGA 50:50 polymer biodegradability is very advantageous for the formulation of a parenteral delivery system and for intra-articular administration, and therefore it seems to be an appropriate choice as a matrix material for the production of this microparticulate system. In this study, to evaluate the influence of the molecular weight of matrix material on microsphere characteristics, PLGA in a 50:50 copolymer ratio was used in two different molecular weight grades (34 000 Da and 88 000 Da). DS-containing PLGA microspheres were prepared by the cosolvent method which is a modification of the solvent-evaporation method. A mixture of methanol and methylene chloride (1.5:5) is used as the organic phase to increase the solubility of the drug. In this process, an increase was noticed in the rate of precipitation of the polymer in the droplet–water interface; thus, the loss of drug into the outer aqueous phase was minimized, ending up with homogenous and smaller particles (Bodmeier, 1994). During the preparation process, stirring time was optimized as 2 h, which was found to be adequate for the complete evaporation of methylene chloride from the medium. PVA and SO were used as stabilizers of the mixture (4:1) in the production of PLGA

Table 2
Measurements of average radioactivity count ratios (mean ± S.D.) at 4 h in ratios of target to non-target areas before and after therapy[a]

Code	n	Pre-treated ± S.D.	Post-treated ± S.D. (3rd day)	Post-treated ± S.D. (17th day)	Post-treated ± S.D. (30th day)
A	5	2.256 ± 0.574	1.974 ± 0.779	2.562 ± 0.946	2.548 ± 0.774
B	5	2.223 ± 0.483	2.711 ± 1.136	2.526 ± 0.310	2.033 ± 0.814
C	5	2.233 ± 0.401	2.296 ± 0.423	2.442 ± 0.979	1.742 ± 0.723

[a] (A) Rabbit treated with DS-containing PLGA microspheres; (B) rabbit treated with blank PLGA microspheres; (C) rabbit treated with DS in buffer solution.

microspheres. These substances and the ratio were found to be critical in preserving the individuality of microparticles (Arshady, 1990; Watts et al., 1990).

When polymeric DS microspheres were evaluated for their yield, no meaningful difference was observed between the two formulations manufactured, and, therefore, it appeared that the molecular weight of the polymer did not have a considerable effect on the yield obtained. On the other hand, the molecular weight of the polymers seemed to be a critical parameter for drug loading, as the loading percentage was determined to be 12.7% for the formulation prepared by the polymer of 34 000 Da, while it was 16.1% for the higher mw polymer (Table 1). Similar results were reported in the literature previously (Çiftçi et al., 1996).

Following the preparation, the particle size of each batch was measured individually and the mean diameters of microspheres were measured as approximately 5–10 μm.

Scanning electron microscopy studies indicated that microspheres showed different surface morphologies depending on the molecular weight of the polymer used in preparation studies (Fig. 2) which could be accepted as an important evidence for the influence of molecular weight and also cosolvent quantity on the porosity (Jeyanthi et al., 1992, 1996). As the molecular weight of polymer increases, the viscosity of the polymer solution will rise and, consequently, it results in solvent extraction before droplet formation — which creates less porous microspheres. When using methanol as a cosolvent, it was observed that methylene chloride extracted slowly and gradually, during droplet formation. This phenomenon also resulted in porous microspheres. Since methanol was used as a co-solvent in combination with methylene chloride in this study, this might be another important factor in the formation of pores on the surface of the microspheres, beside the molecular-weight effect.

The drug release from PLGA microspheres depended on various factors including the polymer composition, polymer molecular weight, drug loading, particle size, porosity and the microstructure of microspheres (Hutchinson and Furr, 1990; Bodmeier, 1994; Jain et al., 1998). The release of DS from PLGA microspheres showed a biphasic pattern. The burst effect in the initial phase of the release might be attributed to DS particles that are not entrapped but adsorbed on the surface of the microspheres. Following this initial phase, the release of DS was slow. In relation to the molecular weight of the polymer and porosity of microspheres, the rate of drug release also showed a difference. In this respect, the result of in vitro release studies revealed that formulation A (mw 34 000 Da), in which the surface of the microspheres appeared to be porous, released the drug faster than formulation B (mw 88 000 Da), whereas the surface seemed to be nonporous. In formulation A, in vitro release of the active substance was almost complete in 84 h, while formulation B released only 69.06% of the drug in the same period. The duration of complete release was 96 h for formulation B (Fig. 3). Hence, polymer molecular weight affected the duration of release to some extent (Wada et al., 1990; Watts et al., 1990; Çiftçi et al., 1996).

Surface morphology of DS-incorporated PLGA microspheres was evaluated after in vitro release experiments. Before conducting the release studies, SEM photographs revealed that microspheres (mw 34 000 Da) had a characteristic porous structure on the surface. An expansion in the pore sizes was noticed following the release (Fig. 2) (Wakiyama et al., 1982; Sato et al., 1988; Iwata and McGinity, 1993; Crotts and Park, 1995; Ghaderi et al., 1996).

Following the evaluation of the formulations and characterization, microsphere formulation prepared using mw 34 000 Da polymer were selected for the intra-articular administration to the knee joints of rabbits, since its release profile was found to be more promising for in vivo conditions.

The labeling efficiency of ^{99m}Tc-HIG complex was determined as 99% by ITLC. ^{99m}Tc-HIG demonstrated arthritic lesions very well (Fig. 4). It was also stable in vivo, as demonstrated by the lack of uptake by the thyroid and stomach. The average radioactivity count ratios at 4 h before and after therapy are given in Table 2.

No meaningful difference was found between DS-containing PLGA microspheres and the empty microspheres used as a control group before treatment and on the 3rd and 17th days after treatment. According to the data at 30th day, there emerges a difference between DS-containing PLGA microspheres and empty microspheres. However, this difference was significant ($P < 0.05$) on the 30th day. One would expect that the A:C ratios would diminish and approach unity as time progressed in group A, and possibly, to some extent, in group C also, indicating the healing effect. However, the A:C ratios remained high in all three groups up to the 30th day.

The A:C values showed a significant variation within groups. This can be attributed to the degree of inflammation induced, and individual variations among animals. Scintigraphic evaluation may not be an ideal method for comparison. The tissue counting of samples with a γ counter might be more sensitive, because in scintigraphy, normal tissues attenuate γ-rays coming from deeper inflammatory tissues.

Another problem is the choice of inflammation model. The present model is antigen-induced chronic arthritis. It might be difficult to treat such inflammation within 30 d. We followed the animals scintigraphically because the animals are not killed at the end of the experiment. It might be better to kill the animals and take tissue sections of arthritic and normal joints for pathologic examination to confirm whether the joint has returned to normal. Moreover, it might be possible to achieve better results if several doses of active substance are used to find the right dose thereof before the scintigraphic examination.

In our previous study (Tunçay et al., 2000), DS-incorporated bovine serum albumin (BSA) microspheres were prepared, and after in vivo evaluation, BSA was found to be a successful carrier matrix of a natural biodegradable kind to provide an extended duration of the active substance, DS, in the knee joints of rabbits. In this study, a regulatory approved, synthetic biodegradable polymer PLGA (50:50) was selected as an alternative carrier matrix of DS for intra-articular administration. However, in respect of the data from in vivo experiments concerning two types of biodegradable polymers, a natural polymer, BSA, seems to be more promising than the synthetic type of biodegradable polymer PLGA for intra-articular administration of a NSAID, DS. Similar results were obtained in previous studies reported in the literature (Ratcliffe et al., 1984).

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