J Mater Sci: Mater Med (2006) 17:1267–1274
DOI 10.1007/s10856-006-0601-0

Release of diclofenac sodium from polylactide-co-glycolide 80/20 rods

Petrus Viitanen · Esa Suokas · Pertti Törmälä · Nureddin Ashammakhi

Received: 13 February 2006 / Accepted: 15 February 2006

Abstract Due to inflammatory reactions complicating bioabsorbable devices, the aim of this study was to develop and characterize bioabsorbable implants with anti-inflammatory drug releasing properties. Polylactide-co-glycolide (PLGA) 80/20 was compounded with diclofenac sodium (DS) to produce rods. Thermal properties were analyzed using differential scanning calorimetry (DSC). Inherent viscosity (η_{inh}) was measured to evaluate the drug effect on the extrude polymer. Drug release measurements were performed using UV-spectrophotometer. Five parallel samples from each type of rods were examined, first at 6 hour intervals, then on daily basis, and later twice a week. DS was released in 110 days from thinner rods and in 150 days from thicker rods. Drug release comprised a starting peak, slow release phase, then a high release phase, and a burst release phase. DSC analysis showed that DS containing rods had crystallinity in their structure. In conclusions, it is feasible to combine PLGA 80/20 and DS by using melt extrusion. Released DS concentrations reached local therapeutic levels, but the release profile was complex and therapeutic levels were not reached all the time.

1 Introduction

Due to the prevalence of problems related to inflammatory reactions such as osteolysis, especially those complicating bone fixation bioabsorbable devices [1, 2], there is a need to develop a new generation of bioabsorbable implants with anti-inflammatory activity that can warrant against these side-effects.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to inhibit osteoclast-like cell formation and this may help to reduce osteolysis[3, 4]. Recently, it has been shown that experimentally induced osteolysis (using UHMWPE particles) in rabbits can be prevented by using NSAID treatment [5]. Although NSAIDs are widely used, their long term systemic use can lead to a number of side effects. By using locally targeted release of NSAIDs to a site of inflammation, we could achieve therapeutic effect with significantly smaller amount of drug, and this could furthermore lower the risk for unwanted side-effects. By local release, we can also get therapeutic drug concentrations to areas that would be hard to reach systemically (poor blood flow etc.). Other benefits of local release are patient comfort and optimized drug concentration and consumption [6]. So far, the studies on local release of NSAIDs have mostly been in the form of injectable microcapsules [7] or devices for delayed release oral administration. To our knowledge, no fixation devices with anti-inflammatory drug releasing properties are available. The promising results of studies made by our group with antibiotic releasing bioabsorbable fixation devices [8] has encouraged us to develop anti-inflammatory releasing fixation devices.

Diclofenac sodium (DS) was selected as the NSAID of choice because it is so far a well known drug and one of the most widely used potent NSAID. DS is one of the most effective NSAIDs for clinical treatment of both inflammation and pain [9, 10]. DS has a relatively high melting temperature (T_m) which allows its processing at high temperatures. DS also has a reasonably high Cox-2 selectivity for a traditional NSAID that might lower the risks of GI side effects. There are promising results of earlier experiments of other research

P. Viitanen · P. Törmälä · N. Ashammakhi (✉)
Institute of Biomaterials, Tampere University of Technology, Tampere, Finland
e-mail: nureddin.ashammakhi@tut.fi

E. Suokas
Linvatec Biomaterials Ltd., Tampere, Finland

groups in combining DS with bioabsorbable polymers [7, 11]. The aim of the current study is to assess diclofenac release from polylactide-co-glycolide (PLGA) 80/20 rods.

Other NSAIDs, especially new COX-2 selective NSAIDs, offer benefits related to less side effects. These new drugs could be a promising research area also in local release applications. Although limited experience of these drugs in long-term use was the main reason that we decided to do this pilot study with a traditional NSAID. Reports have also emerged on how the so called COX-2 specific drugs have effects on both COX-1 and COX-2 [12, 13]. In addition, many of these drugs are relatively new (celecoxib 1998, rofecoxib 1999) as compared to nonspecific NSAIDs and one (diclofenac 1974) DS remains one of the best as regards relative specificity among the traditional NSAIDs [12, 13].

DS has shown to have good tolerability. It has a long clinical history and it is widely used. Because of its relatively high melting point, it is also suitable to be used with melt molding techniques of bioabsorbable polymers. All these factors in combination made DS the candidate drug of choice to include in studied implants.

2 Materials and methods

2.1 Materials

A copolymer of lactide and glycolide (PLGA 80/20) (Purasorb®, Purac biochem bv Gorinchem, Netherlands) was used as a matrix polymer. Intrinsic viscosity of the batches used in compounding trials C-1 and C-2 was 6.2 and 7.2 dl/g respectively. Diclofenac sodium (DS) (Sigma-Aldrich, Espoo, Finland) was used as an anti-inflammatory agent.

2.2 Methods

2.2.1 Compounding PLGA and DS

The polymer granules and DS in powder form were compounded to billets using a small scale laboratory extruder. Two compounding trials were performed in this study. In the first trial (C-1) Ø3 mm die and in the second trial (C-2) Ø7 mm die were used. The polymer and the drug were first separately dried in a vacuum oven (Binder VD 115, WTB Binder, Germany) and then mechanically mixed together using an electrical blender (Retsch Grindomix GM200, Retsch GmbH & Co. KG, Haan, Germany). In C-1, the materials were mixed at the speed of 2000 rpm for 20 s, repeating the procedure four times for each patch. In C-2, the materials were first mixed for 15 s using 4000 rpm, and then once for 20 s using 2000 rpm. The mixed drug and the polymer were put again to the vacuum oven to dry. In C-1, the heating procedure in drying (80C for 8 hours) was used to remove all remaining moisture. No heating was used in C-2, because the materials were kept in the vacuum oven longer, due to the delay in processing. PLGA 80/20 rods compounded with three different amounts of drug were manufactured by means of melt extrusion: 8 wt-% in C-1, 4 wt-% and 2 wt-% in C-2. Neat PLGA 80/20 rods were also manufactured.

In C-1, a water-cooled cooling plate and a manually controlled drawing belt were used, while cooling with pressurized air. Raw materials were fed by the feeding device through the hopper into the extruder barrel. The feed-rate was measured before the compounding to be 11.4 g/min. When the rod came out from the extruder, it was manually drawn onto the drawing belt. The speed of the belt was adjusted manually during the process to control the rods diameter. Extrusion parameters were adjusted during the extrusion process.

In C-2, no cooling plate was used but only cooling with pressurized air. A laser measuring unit together with a take-off device unit were used for better optimization of the billet diameter. Compounded rods of C-1 were 2.2–2.4 mm in diameter, and those of C-2 were had a diameter in range 6.2–7.4 mm.

2.2.2 Determination of thermal properties

Thermal properties of the DS containing PLGA specimens were analyzed using differential scanning calorimetry (DSC). Samples were weighed using Mettler AT621 precision scale (Mettler Instrumente AG, Grefenzee, Germany) in aluminum pans. Tests were performed using TA Instruments Q1000 Differential Scanning Calorimeter (TA Instruments Ltd., New Castle, DE, USA). As a purge gas, nitrogen was used. The scanning procedure for PLGA consisted of two phases. In the first stage the sample was heated from 10 to 200°C at the rate of 20°C/min and then cooled from 200 to 10°C at the rate of 50°C/min to eliminate crystallinity . In the second stage the sample was again heated from 10 to 200°C (20°C/min). The melting point (T_m) and the heat of fusion (ΔH) were determined from the first heating. The glass transition temperature (T_g) was determined from the second heating. Samples of all compounded rods were prepared and analyzed. Pure DS was analyzed, using two parallel samples to determine the melting point and other possible thermal reactions. Additionally, possible reactions between the two components processed under high temperatures was assessed by preparing samples of PLGA 80/20 granules and DS powder (about 20 wt-%) combined.

2.2.3 Determination of inherent viscosity

Dilute solution viscometry is a convenient method to indicate the molecular weight of polymers. Each sample was prepared diluting 20 ± 0.8 mg of material into chloroform in 20 ml vials and then left to dissolve for 2–3 days. After that, the bottle was filled to the containing mark with chloroform and then stirred. The resulting concentration of the sample was 10 mg/ml (0.1%). Ubbelohde viscosimeter was used (Schott, Germany) and inherent viscosity (η_{inh}) was calculated. Two parallel samples were used.

2.2.4 Determination of drug release

Drug release measurements were performed by using UV-spectrophotometer (UNICAM UV 540, Thermo Spectronic, Cambridge, UK). Samples were weighed precisely and then placed in vials filled with 10 or 40 ml of phosphate buffer solution (KH_2PO_4+ NaOH, pH 7.4 ± 0.02), depending on the sample weight and drug concentration. Bottles were kept in an incubator at 37°C. For every measurement, five parallel samples were investigated.

The drug release profile for each sample was determined by measuring the concentration of released DS in a phosphate buffer for suitable intervals. Bottles were emptied into test tubes and the concentrations were measured with UV. Bottles were always refilled with fresh buffer solution. During the first days, measurements were made more frequently (6 h, 18 h, 1 d, 2 d etc.) to determine the time in which therapeutic daily concentrations of DS were reached. Based on the results of early measurements that were made, the measurement points were then defined to be less frequent, usually once or twice a week. Daily released concentrations were determined by dividing the amount of released drug with the days of hydrolysis since the last measurement. From these results, a drug release profile specific for each type of sample was obtained.

2.2.5 Characterization of microstructure

Scanning electron microscopy was used to analyze the microstructure of the drug-polymer composite. SEM imaging was carried out using JEOL T100 scanning electron microscope (JEOL Ltd. Tokyo, Japan). Samples were cooled in liquid nitrogen and cut. Before SEM imaging, the samples were sputtered with gold using Edwards S150 sputter coater.

2.2.6 Measuring the drug content

Actual drug content inside the rods was measured because it was presumed that the rods had a smaller amount of drug inside the structure than the amount originally mixed in. Drug content was measured from ten randomly selected rod locations for every composition. 20 mg samples of the rods were dissolved into a mixture of chloroform and ethanol (chloroform:ethanol 1:4) in 20 ml vials. This solvent was used because DS is not soluble in chloroform and highly soluble in ethanol. PLGA 80/20, on the other hand, is soluble in chloroform but not in ethanol. Samples were first left to dissolve overnight in chloroform so that the polymer was completely dissolved. After that, ethanol was added and the vials were filled to the containing mark with chloroform. Concentrations of DS were measured from the solutions with UV-Vis spectrophotometer, using a standard curve especially determined for the used solvent. Drug amount in the rods was then calculated by dividing the amount of drug in the solution by the mass of the sample.

3 Results

3.1 Compounding

In C-1, compounding DS (8 wt-%) to PLGA 80/20 rods of 2.9–3.2 mm in diameter was achieved (Fig. 1). The surface of the rods was grainy and possibly non-melted polymer particles were seen in the structure. In C-2, PLGA 80/20 rods containing 0, 2 and 4 wt-% of DS were produced. Problems with surface quality were encountered in C-2 rods, which were similar to those seen in C-1 rods. Microscopically, areas of possible non-melted polymer material that contained no drug were seen in all DS containing compounded PLGA 80/20 rods.

3.2 Thermal properties (DSC)

Melting peak for pure DS was indicated at 296°C. In temperatures above that a few peaks were seen indicating decomposition of the drug. Reactivity between the PLGA 80/20 and DS in heating was not seen in DSC curves. Nevertheless

Fig. 1 Compounded rods of the first and second compounding trial. 1: 1CO PLGA 80/20 + DS 8 wt-% (Ø ∼3 mm), 2: 2CO PLGA 80/20 (Ø ∼6 mm), 3: 2CO PLGA 80/20 + DS 4 wt% (Ø ∼6 mm), 4: 2CO PLGA 80/20 + DS 2wt-% (Ø ∼6 mm)

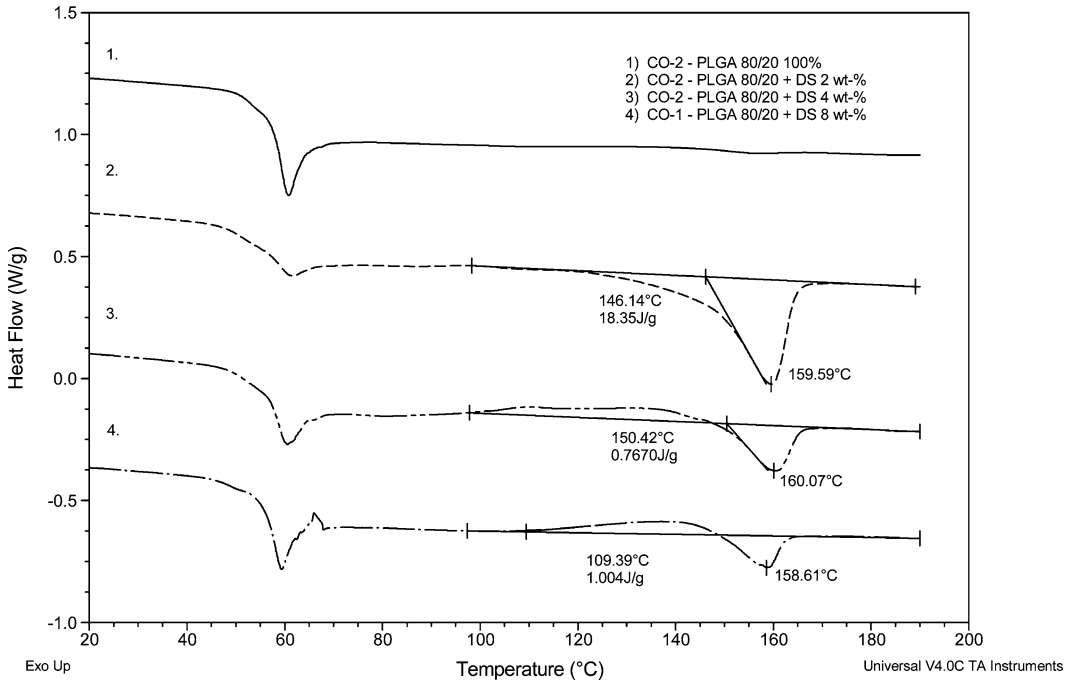

Fig. 2 DSC curves from the first heatings of compounded PLGA rods containing: (1) no drug, (2) 2 wt-% diclofenac sodium (DS), (3) 4 wt-% DS, (4) 8 wt-% DS

the color of the combined polymer and drug sample was seen to change to dark brown when heated above 220˚C. This should not a problem since the processing temperatures were always below 200˚C. Resulting DSC curves of the manufactured rods analyzed of the first and second heating are presented in Figs. 2 and 3. Remaining crystallinity in the rods after compounding can be seen in Fig. 2. There was no or very little crystallinity in neat PLGA 80/20 rods (1) left ($\Delta H \sim 0$). In all DS containing rods, an endothermic melting peak can be seen indicating crystallinity. Heat of fusion

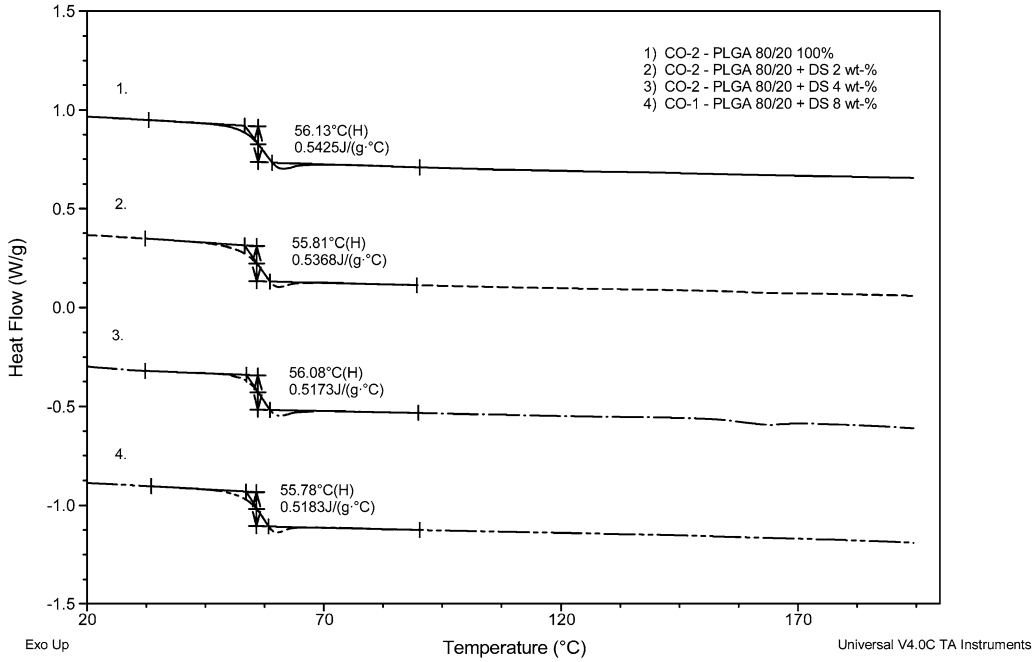

Fig. 3 DSC curves from the second heatings of compounded PLGA rods containing: (1) no drug, (2) 2 wt-% diclofenac sodium (DS), (3) 4 wt-% DS, (4) 8 wt-% DS

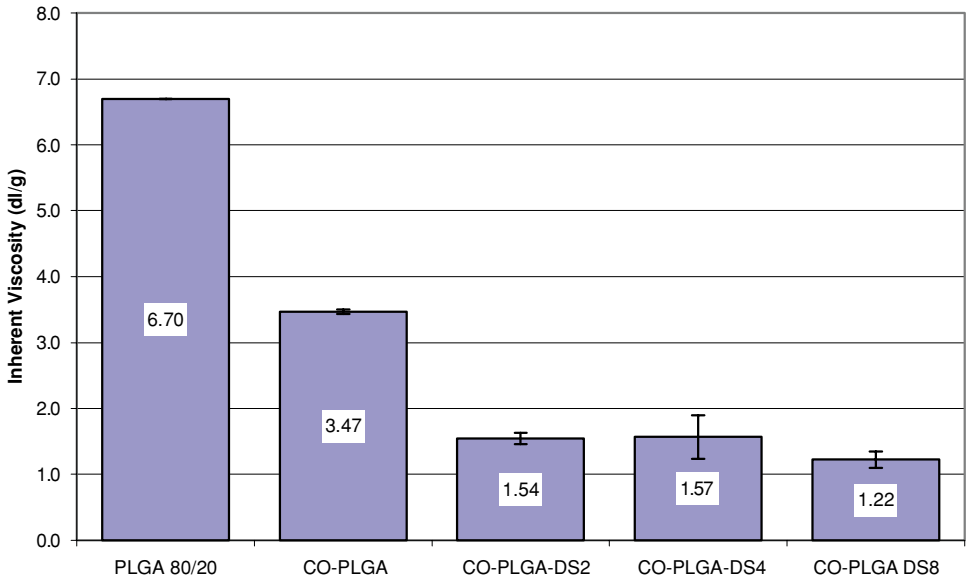

Fig. 4 Inherent viscosities: "PLGA 80/20" is the original polymer resin used. "CO-PLGA" is the extruded neat PLGA 80/20 rod. Rest of the bars are the compounded 2, 4 and 8 wt-% diclofenac sodium containing PLGA 80/20 rods

of the exothermic peaks indicating formation of crystallinity during the heating were subtracted from the calculations of the heat of fusion (ΔH). For the PLGA 80/20 rods containing 2 wt-% DS (2); $\Delta H = 18.35$ J/g, 4 wt-% DS containing rods (3); $\Delta H = 0.7670$, 8 wt-% DS containing rods (4); $\Delta H = 1.004$.

3.3 Inherent viscosity

Calculated medians and standard deviations of the two measurements are presented in Fig. 4. When unprocessed (PLGA 80/20 granules used in C-1) and processed (CO-PLGA) polymer are compared, it can be seen that the viscosity decrease caused by the extrusion process is about 48%. Furthermore, the addition of DS also reduces viscosity.

3.4 Drug release

C-1 rods: Daily drug release (μg/ml/day) of the PLGA 80/20 rods compounded in C-1 and containing 8 wt-% of DS is presented in Fig. 5. After the start burst that occurred at approximately 6 hours, the release rate stays very low during the

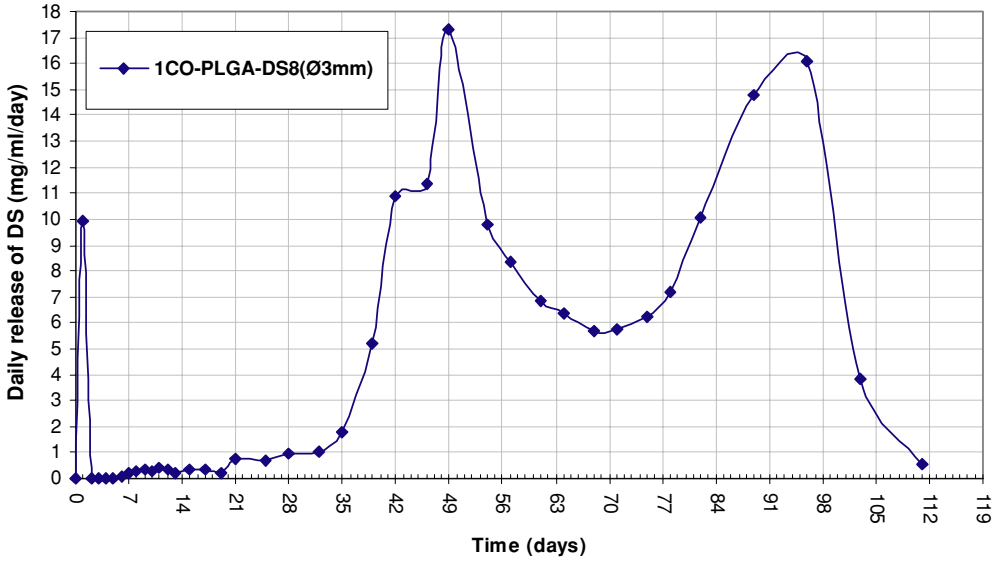

Fig. 5 Daily released concentration of diclofenac sodium (DS) released from PLGA 80/20 rods of the first compounding trial (C-1). Rods were mixed with 8 wt-% of DS and were ~3 mm in diameter

Table 1 Measured drug contents of the compounded rods manufactured at the first and second compounding trials (COT-1, COT-2)

	Rod type	Mixed drug content (%)	Average drug content measured (%)	Max. drug content measured (%)	Min. drug content measured (%)	Standard deviation
1	CO-PLGA-DS2	2	1.6	1.9	1.4	0.14
2	CO-PLGA-DS4	4	3.0	3.3	2.6	0.22
3	CO-PLGA-DS8	8	5.8	7.1	4.9	0.85

first 20 days. During days 21–35, the release rate remains at a relatively constant level of about 1 μg/ml/day. During days 35–70, there is an area of high release that reaches a peak value of approximately 17 μg/ml/day. During days 70–110, an end peak was seen resulting from partial degradation of the polymer matrix. During the end peak, all remaining drug was released and the daily release reached a maximum value of approximately 16 μg/ml/day. The release was completed in about 112 days.

C-2 rods: Daily drug release (μg/ml/day) of the PLGA 80/20 rods compounded in C-2 and containing 2 and 4 wt-% of DS is presented in Fig. 6. Start burst is seen at 6 hours. The Intensity of the start burst peak is dependent on the amount of drug in the polymer and possibly also on rod dimensions. After the start burst, the release rate drops sharply to almost zero and stays very low for the first 40 days. The level of daily release remained approximately stable during the period from day 65 to day 105 being 1–2 μg/ml for 4 wt-% DS and 0.5–1 μg/ml for 2 wt-% DS containing rods. During days 110–145, an end peak is seen in both 2 and 4 wt-% DS containing rods, after which the release stops. The release was complete in approximately 150 days. Generally, the release profile of both 2 and 4 wt-% DS containing rods was similar. There was only a difference in the intensity of the release, depending on the amount of drug in the polymer matrix. Release profiles of C-1 and C-2 rods were quite different most probably because of the different rod dimensions.

3.5 Characterization of microstructure

The micrographs (Fig. 7) suggest that drug particles lie throughout the matrix of the rods. Nevertheless, areas where agglomerated drug particles exist in a separate phase are seen.

3.6 Actual drug content

Actual drug content of the DS containing rods (Table 1) is lower than the content originally mixed. A fraction of the drug is always stuck on the metal surfaces of the blender and extruder. The standard deviation and differences between the maximum and minimum drug contents suggest that mixing of the drug into the polymer has been better in the second compounding trial (C-2) (1, 2) than in the first compounding trial (C-1) (3).

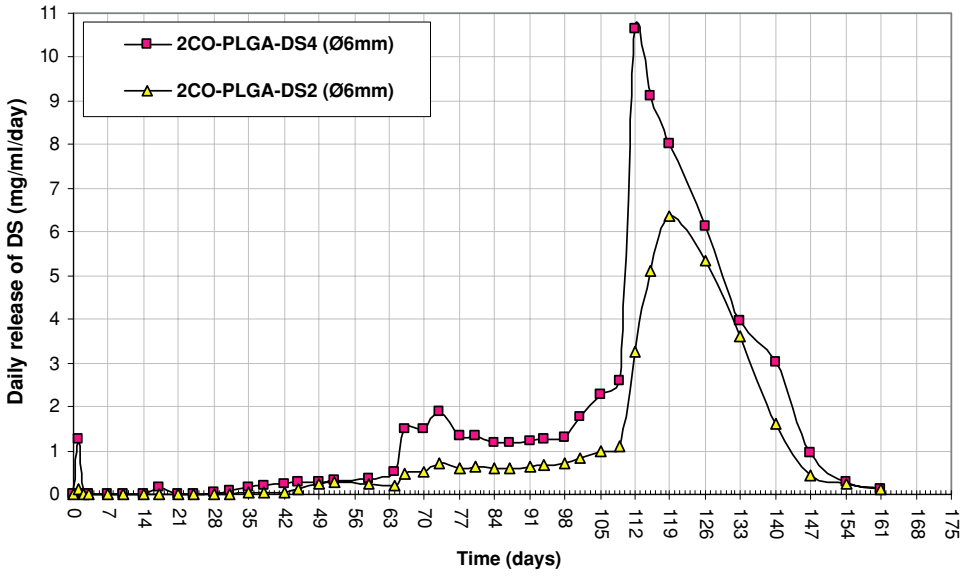

Fig. 6 Daily released concentration of diclofenac sodium (DS) released from PLGA 80/20 rods of the second compounding trial (C-2). Rods were mixed with 4 wt-% (2CO-PLGA-DS4) and 2 wt-% (2CO-PLGA-DS4) of DS and were ~6 mm in diameter

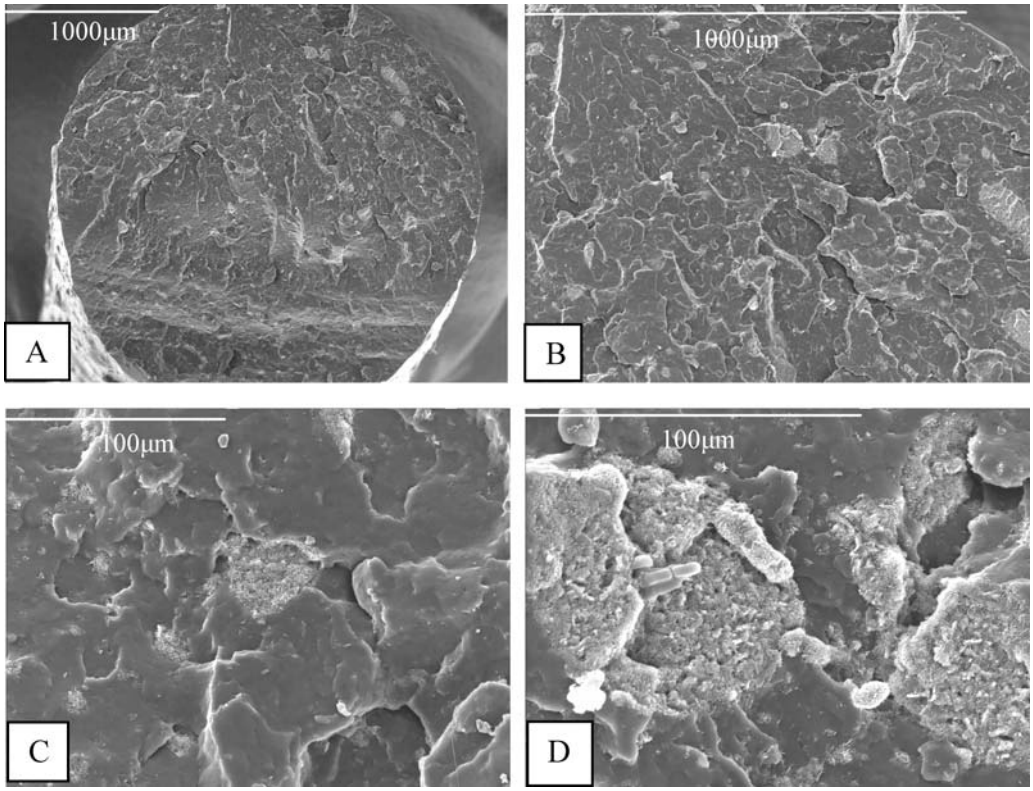

Fig. 7 SEM micrographs of fracture surface of compounded PLGA 80/20 rods containing 8 wt-% of diclofenac sodium (DS). Samples were cooled in liquid nitrogen and broken along cross direction: A: 35×, B: 100×, C: 500×, D: 750×

4 Discussion

In a recent experimental work on rabbits the use of an NSAID, celecoxib, has been shown to be effective in treatment of experimentally induced osteolysis, using ultra-high-molecular-weight-poly-ethylene (UHMWPE) particles [5]. DS in local sustained release applications has been investigated in earlier studies [7, 14].

DS is classically administered systemically. However, local tissue levels do not achieve the same levels as those in the plasma. In addition, there is a delayed achievement of appropriate levels at the target site [9]. Systemically administered DS is thus associated with high plasma levels that may damage other tissues inadvertently. Side effects of DS and other NSAIDs are related to the high doses affecting gastric and renal function. Local delivery would satisfy local need of the drug in the target site, thus lowering the risk for side effects.

Concerning the methods used in the current study, there was an overall problem with the quality of DS containing PLGA 80/20 rods. The surface was grainy and areas of unmelted polymer were found on the rods. The problem was worse with the rods containing 2 wt-% of DS, which could not be explained.

The daily released concentrations of DS were thought to be at a local therapeutic level for most of the time of the release period. As a reference lower level we used 0.12 ug/ml as being the concentration of DS in synovial fluid after 12 hours from oral administration of 75 mg DS [9]. No reference of toxic level of DS in humans was found in the literature. In the few cases of overdose concentrations of 60 ug/ml in plasma have been measured. Therefore, 60 ug/ml was considered as upper therapeutic limit [9, 13]. High concentrations at the start burst were most likely due to the dissolution of drug particles at the very surface of the rod that were not completely surrounded by the polymer matrix [15]. The end peak where all remaining drug was released at a high rate resulted from partial polymer degradation and could be a problem. Nevertheless, no toxic concentrations were reached at any time of the release period [9]. As demonstrated in preliminary cell tests, DS released from the compounded PLGA 80/20 rods was shown to be bioactive (unpublished data). To overcome these issues, further research with the polymer and manufacturing techniques is necessary. Other bioabsorbable polymers could be experimented. By a longer degradation time of the polymer, we might be able to sustain the release rate more stable throughout the time of release. Adding non-polymeric substances, such as bioceramic particles, might also affect the microstructure in a way that faster and more stable absorption of water into the matrix could be achieved. This would probably lead to more an even drug release profile.

Dilute solution viscometry measurements showed that inherent viscosity of the neat polymer rods is higher than that of

DS containing rods. The reason for viscosity decrease when the drug is present is probably that the particles have grinding effect during the extrusion process. There may also be other reasons such as chemical reactivity and differences in heat absorption properties.

Dry blends of the polymer and drug were quite uneven because of poor attachment of the drug particles onto the polymer granules. This made the smooth and even feeding of materials into the extruder difficult. Poorly mixed dry blends may result in heterogeneous morphology, i.e., a phase separation in melt mixing. However, phase separated microstructure might even be advantageous as regards to the release of the drug. One solution to this might be by grinding the polymer granules into smaller particles. Preliminary studies have shown this to be beneficial in solving this problem.

DSC measurements showed that DS tends to induce crystallinity when compounded with the PLGA 80/20 using melt-extrusion. It is possible that the trans-crystallization is induced by drug particles that act as the nuclei of crystals. According to the literature, crystallinity may have a delaying effect on drug release from the polymers. Water cannot penetrate into crystalline regions and thus the drug is blocked inside the structure [16].

5 Conclusions

It is feasible to combine diclofenac sodium (DS) with PLGA 80/20 and, by the use of melt extrusion, to produce rods that can release DS over for 110–150 days. During the first weeks, DS release reached local therapeutic levels but was probably under therapeutic levels after the "jump-start"-peak. The release profile is more dependent on the rod dimensions than on drug concentration. Polymer properties such as viscosity and crystallinity are affected by adding the drug. Further development and optimization of the processing methods and drug release properties are required.

Acknowledgments Research funds from The Technology Development Center in Finland (TEKES, 90220), The European Commission (European Union R&D Project QLRT-2000-00487, EXPERTISSUES Project NMP3-CT-2004-500283) and The Academy of Finland (Project 37726) are greatly appreciated. The authors thank Ms. Hilkka Losoi and Ms. Mari Keijola for administrative assistance.

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