Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Controlled Release 112 (2006) 293–300
journal of controlled release
www.elsevier.com/locate/jconrel

Elevated temperature accelerated release testing of PLGA microspheres

Banu S. Zolnik [a], Pauline E. Leary [b], Diane J. Burgess [a,*]

[a] Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, United States
[b] Purdue Pharma L.P. Ardsley, NY 10502, United States

Received 12 October 2005; accepted 23 February 2006
Available online 27 April 2006

Abstract

Drug release from four different poly(lactic-co-glycolic) acid (PLGA) microsphere formulations was evaluated under "real-time" (37 °C) and accelerated release testing conditions of elevated temperature (45, 53, 60 and 70 °C) and increase in flow rate (4–35 ml/min) using United States Pharmacopeia (USP) apparatus 4. Formulation 5 K (composed of low Mw PLGA) exhibited diffusion-controlled kinetics in "real-time". Whereas, formulations 25 K, 28 K and 70 K (composed of medium and high Mw PLGA) followed erosion-controlled kinetics at 37 °C. Temperature-induced degradation of the microspheres was studied by monitoring drug release rates, change in molecular weight and morphological changes. Drug release rates at elevated temperature were used to predict "real-time" release applying the Arrhenius equation. The energy of activation for dexamethasone release from PLGA microspheres was calculated as 19.14 kcal/mol. Molecular weight change measured by gel permeation chromatography followed first order kinetics for both "real-time" and accelerated release. All four formulations exhibited morphological changes (such as surface pore closing and geometry change) at elevated temperature with consequent reduction in burst release.

Keywords: Biodegradable polymers; Dexamethasone; Mechanism of release; Diffusion-controlled; Polymer degradation

1. Introduction

Poly(lactic-co-glycolic) acid (PLGA) and other biodegradable polymers are used extensively in microsphere and other controlled drug delivery implantable devices. Release kinetics from PLGA and other biodegradable polymers are controlled by diffusion, erosion or a combination thereof [1], and are dependent on the polymer (Mw, copolymer ratio and crystallinity) [2–5], drug properties [6–8], as well as the device characteristics (preparation conditions, particle size, morphology, porosity and drug loading) [9–14] and the dissolution conditions [10,15]. Drug release from PLGA microspheres in "real-time" (37 °C) typically shows a triphasic profile: (1) an initial burst release of surface and pore associated drug, (2) a lag phase until sufficient polymer erosion has taken place and (3) a secondary burst with approximately zero order release kinetics [16]. The initial burst release is controlled by diffusion, whereas the lag phase and secondary burst phase are dependent on polymer erosion as well as diffusion.

Drug release profiles from PLGA microspheres can range from days to months and, consequently, there is a need for accelerated release testing of such systems for quality control purposes as well as to aid in formulation design. Ideally, drug release from accelerated and "real-time" tests should follow the same release mechanism with a 1:1 correlation. However, since the accelerated tests require extreme conditions (temperature, pH, etc.) to achieve rapid release, it is possible that the release mechanism may change. Nevertheless, "real-time" and accelerated release profiles should show a minimum of a rank order relationship between different formulations [17]. In a recent AAPS-EUFEPS workshop report on "Assuring Quality and Performance of Sustained and Controlled Release Parenterals", it was suggested that specifications for accelerated testing include an early time, mid-point and >80% cumulative release for comparison with "real-time" studies [17]. It was also suggested that prediction of "real-time" release can be achieved by comparing the time to terminal plateau of accelerated release for different formulations.

* Corresponding author.
E-mail address: d.burgess@uconn.edu (D.J. Burgess).

0168-3659/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2006.02.015

Accelerated drug release from PLGA can be achieved by: increase in polymer degradation rate via acid or alkali catalyzed hydrolysis; addition of surfactants to enhance drug diffusion; or increase in temperature which enhances polymer mobility and therefore drug diffusion [18–22]. It has been reported that, at temperatures near the glass transition temperature (Tg) of the polymer, drug diffusion coefficients may increase by as much as three orders of magnitude [23]. Other conditions that can result in accelerated polymer degradation and hence more rapid release rates include addition of a co-solvent and radiation [24,25].

There are few literature reports on accelerated drug release testing from PLGA microspheres and other delivery devices. Shameem et al. [26] have investigated leuprolide release from PLGA microspheres at elevated temperature, low pH, different buffer concentrations and in the presence of surfactant for two different formulations (28,000 and 8600 polymer Mw). These authors were able to achieve an approximately 1:1 correlation between "real-time" and accelerated release data by optimization of the above accelerated testing conditions. Aso et al. [20] investigated the effect of temperature on drug release from PLA systems (microspheres and discs) and reported that no significant release occurred below the Tg over the experimental period. However, at temperatures above the Tg, drug release rates increased with increase in temperature. Hakkarainen et al. [21] investigated degradation of PLA and PLGA polymers of different co-polymer ratios and molecular weights at 37 °C and 60 °C with respect to molecular weight change, mass loss and formation of lactic and glycolic acids. They reported that the degradation profiles were similar at both temperatures and only the rate was increased at elevated temperature.

The effects of different temperature conditions on in vitro drug release kinetics and on the physico-chemical properties of drug loaded microspheres are reported here. The microspheres were characterized using gel permeation chromatography, differential scanning calorimetry and field emission scanning electron microscopy. A relationship between "real-time" and accelerated release was developed for different microsphere formulations.

2. Materials and methods

2.1. Materials

Poly(D,L lactic-co-glycolic acid) (PLGA) polymers, PLGA Resomer RG503H 50:50 (Mw: 25,000), PLGA Medisorb 50:50 DL 2.5A (Mw: 28,000) and PLGA Medisorb 65:35 DL (Mw: 70,000) were gifts from Boehringer-Ingelheim, Astra Zeneca and Purdue Pharma, respectively. PLGA Medisorb 50:50 DL 1A (Mw: 5000) was purchased from Alkermes. All PLGA polymers studied have carboxylic acid end groups with the exception of PLGA Medisorb 65:35 DL, which is end-capped. Methylene chloride and tetrahydrofuran (Optima grade) were obtained from Fisher Scientific (Pittsburgh, PA). Dexamethasone and poly(vinyl alcohol) (PVA) were obtained from Sigma (St. Louis, MO).

2.2. Methods

2.2.1. Preparation of microspheres

An oil-in-water (o/w) emulsion solvent extraction/evaporation technique was used for dexamethasone microsphere formulation. 2 g of PLGA was dissolved in 8 ml of methylene chloride and 200 mg of dexamethasone was dispersed in this solution using a homogenizer at 10,000 rpm for 1 min. This organic phase was added slowly to 40 ml of a 1% (w/v) aqueous poly(vinyl alcohol) (PVA) (average molecular weight 30,000–70,000) solution and homogenized at 10,000 rpm for 3 min. This emulsion was added to 500 ml of a 0.1% (w/v) aqueous PVA solution and stirred at 250 rpm under reduced pressure for 6 h at 25 °C. The resulting microspheres were filtered (Durapore Membrane Filter, 0.45 μm, Fisher Scientific, Pittsburgh, PA), washed three times and vacuum dried for 24 h.

2.2.2. Characterization of microspheres

2.2.2.1. High performance liquid chromatography (HPLC). The concentration of dexamethasone was determined using HPLC. The HPLC system consisted of a Constametric 4100 pump (Thermoseparation), an automatic sample injector (Bio-Rad) and a UV absorbance detector (Bio-Rad) set at 242 nm. The mobile phase consisted of acetonitrile/water/phosphoric acid (30:70:0.5 v/v/v). The analytical column was a Nova-Pak® C_{18} (9 mm × 150 mm) (Millipore Corp., Waters, Milford, MA). The flow rate was set at 1 ml/min. The retention time of dexamethasone was 5 min. The chromatograph was analyzed by PeakSimple Chromatography System (Model 203, software 3.29, SRI instruments, Torrance, CA) [27]. This method is a stability indicating HPLC assay.

2.2.2.2. Drug loading. 10 mg of microspheres were dissolved in 10 ml of tetrahydrofuran (THF) filtered (Millex-HV, 0.45 μm, Fisher Scientific, Pittsburgh, PA) and analyzed by the HPLC method, described above for dexamethasone content. Encapsulation efficiency was determined as reported by Fu et al. [28]:

$$= (\text{experimental drug loading}/\text{theoretical drug}) \times 100\%$$

All the measurements were conducted in triplicate and the mean values and standard deviations are reported.

2.2.2.3. Differential scanning calorimeter (DSC). Samples were analyzed using a TA Instruments 2920 DSC. Samples were heated to 150 °C and cooled to −30 °C at a rate of 5 °C/min and the second cycle was used to determine the glass transition temperature (Tg). Samples were analyzed in aluminum pans with pinhole lids.

2.2.2.4. Gel permeation chromatography (GPC). The molecular weight of the PLGA microspheres was determined by GPC (Waters) with an evaporative light scattering detector (ELSD, Polymer Laboratories PL-ELS 1000). Four columns, Jordi Flash (high-speed) were connected in series. The mobile phase was THF with a flow rate of 3 ml/min at 40 °C. Microspheres (10 mg) were