Polymer Degradation and Stability 119 (2015) 228–241

Contents lists available at ScienceDirect

Polymer Degradation and Stability

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

Investigation of factors influencing the hydrolytic degradation of single PLGA microparticles

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ARTICLE INFO

Article history:
Received 11 March 2015
Received in revised form
15 April 2015
Accepted 27 April 2015
Available online 12 May 2015

Keywords:
Polymer degradation
PLGA
Controlled release
ATR-FTIR spectroscopic imaging
Gamma-irradiation
Degradation rate

ABSTRACT

Poly lactide-co-glycolide (PLGA) is an important polymer matrix used to provide sustained release across a range of active pharmaceutical ingredients (APIs) and works by hydrolytic degradation within the body, thereby releasing entrapped drug. Processing and sterilisation can impact on the morphology and chemistry of PLGA therefore influencing the hydrolysis rate and in turn the release rate of any entrapped API. This paper has looked at the effect of supercritical carbon dioxide ($scCO_2$) processing, gamma irradiation, comonomer ratio and temperature on the hydrolysis of individual PLGA microparticles, using a combination of Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) imaging, Scanning Electron Microscopy (SEM), Differential Scanning Calorimetery (DSC) and Gel Permeation chromatography (GPC) to facilitate a better understanding of the physiochemical factors affecting the hydrolysis rate. This work has shown that $scCO_2$ processing influences hydrolysis rates by increasing the porosity of the PLGA microparticles, increasing the lactide comonomer ratio decreases hydrolysis rates by reducing the hydrophilicity of the PLGA microparticles and increasing the gamma irradiation dose systematically increases the rate of hydrolysis due to reducing the overall molecular weight of the polymer matrix via a chain scission mechanism. Moreover this work shows that ATR-FTIR imaging facilitates the determination of a range of physicochemical parameters during the hydrolysis of a single PLGA microparticle including water ingress, water/polymer interface dimensions, degradation product distribution and hydrolysis rates for both lactide and glycolide copolymer units from the same experiment.

1. Introduction

As a result of recent research in molecular and cell biology to meet clinical needs, the interest in biologics (a variety of therapeutics such as vaccines, recombinant proteins and peptides, genes, viruses and synthetic tissues) has intensified leading to a strong market growth, consequently currently hundreds of recombinant proteins and peptides are in the pipeline for U.S. Food and Drug Administration (FDA) approval [1]. In particular controlled release protein formulations in the form of microparticles, which release protein upon degradation of the carrier matrix, are being developed at a rapid rate as they change the period between injections from a few days to several weeks, enhance the protein therapeutic effect, and therefore noticeably increase patient compliance [2–4].

Although there are numerous candidate biodegradable polymers for carrying and releasing biologics in a controlled manner in the form of microparticles, there are few that are FDA approved due to their limited biocompatibility within the human body [5,6]. Poly (lactide-*co*-glycolide) (PLGA), a random copolymer of poly(glycolic acid) (PGA) and poly(lactic acid) (PLA), is an FDA approved biodegradable synthetic polyester that is physically stable and highly processable [6–9]. Hence, PLGA, in the form of microparticles, has been the most studied carrier matrix for macromolecules such as proteins, DNA, RNA, vaccines and peptides for treatment of several important diseases including cancer [10–13]. Another advantage of PLGA is that its degradation *in vivo*, which governs the drug release, is controllable mainly by choice of comonomer ratio (i.e. lactide to glycolide (*L/G*)), microparticle morphology and to a lesser extent molecular weight; it also yields lactic and glycolic acids, which are biocompatible and rapidly cleared from the body via the renal system [5].

In order to produce microparticles that release drugs effectively, the drug needs to be mixed with in the polymer(s) homogenously and this is highly dependent on liquefication of the polymer(s)

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http://dx.doi.org/10.1016/j.polymdegradstab.2015.04.025

during the formation of particles. Common methods for producing sustained release drug delivery systems, which differ mainly in how they are liquefied include; spray drying [14], emulsification processes (single and double) [13] and more recently using a supercritical fluid-based Particles from Gas Saturated Solution (PGSS) process in a novel way, i.e. CriticalMix™. Unlike the aforementioned methods, CriticalMix™ has the advantage in that it works in the absence of organic solvents and the polymer and drugs do not need to be soluble in $scCO_2$. However the polymer does need to be sufficiently plasticised by $scCO_2$, which can be achieved at moderate temperatures (typically <40 °C) and pressures (<150 bar) that have no adverse effect on protein stability within controlled release PLGA/PLA formulations [15].

It is well known that polymer morphology has a role in the degradation of microparticles and that $scCO_2$ creates porosity, therefore the first aim of this work was to characterise the physiochemical effects of $scCO_2$ processes on the *in vivo* degradation of a PLGA 50/50 microparticle using ATR-FTIR imaging for the first time.

Understanding polymer degradation is critically important for modifying the performance of any biodegradable polymeric drug delivery system. PLGA degrades via chain scissions of ester bond linkages in the polymer backbone by hydrolytic attack of water molecules [16,17]. Factors effecting the hydrolytic degradation of PLGA devices in the form of microspheres [18,19], porous scaffolds [20], dense films [21] and cylinders [22,23] have been widely studied. In recent work by the authors it has been shown that FTIR imaging in ATR mode using a focal plane array detector coupled with multivariate analysis has provided visual evidence of morphological and physiochemical changes during hydrolysis of a PLGA microparticle *in situ*. Quantitative information including hydrated layer size surrounding the particle, width of the particle and perhaps more importantly the degradation rate of individual glycolic and lactic units in real time, the latter of which was reported to be calculated more accurately using the novel non-linear curve fitting procedure compared to traditional peak height image analysis [24]. Using the same experimental procedure and relevant image analysis methods developed by the authors the degradation characteristics of a family of PLGA microparticles with L/G molar compositions of 100/0, 75/25 and 50/50 in water are reported herein by calculating the hydrolytic degradation rate of the individual lactic and glycolic acid units and relating to morphological changes.

Biodegradation of a PLGA microparticle studied by the authors using FTIR imaging in ATR mode was observed to occur through a hydrolytic chain cleavage mechanism and the rates of polymer degradation were similar for both the surface and bulk microparticles [24]. Since degradation of the PLGA microparticles generates acidic monomers, μpH distribution mainly within the bulk has been one of the important questions concerning protein stability within degrading PLGA microparticles. Several groups have quantified μpH in PLGA matrices by several methods including; nuclear magnetic resonance (NMR) [25], electron paramagnetic resonance (EPR) [26], and confocal fluorescence microscopy using various dyes as labels [27–29]. Although reported results on pH distribution in degrading polymers vary, it is well established that the acidic monomer distribution mainly depends on the morphology and shape of the microparticle which is, in turn, mainly governed by the production method [27]. Herein a single PLA microparticle has been evaluated within a 640 μm × 640 μm field of view using FTIR imaging in the ATR mode and the inherently rich chemical signal of mid-IR vibrations without any chemical label or dye for the first time.

Jordan et al. [30] showed that novel sustained release PLGA/PLA microparticles formulations containing human growth hormone (hGH) could be prepared by the CriticalMix™ process with 100% encapsulation efficiency. This formulation has been shown to demonstrate >2 weeks longer efficacious hGH release compared to a daily injection of soluble hGH *in vivo* in rats and monkeys and *in vitro* [3]. This same formulation has been investigated for its real time release using ATR-FTIR imaging for the first time [31]. The study also evaluated the effect of γ-irradiation, a well-established method to sterilise polymeric microparticle drug delivery systems before use, on their physical and chemical structure. The release mechanism of hGH was elucidated with respect to the release kinetics changing as a result of modifications to the microparticle morphology and chemistry during γ-irradiation. This finding raised important questions regarding their degradation. Interestingly, spectroscopic changes resulting from γ-irradiation on bulk PLGA 50/50 were not detected using FTIR spectroscopy, however the observation that protein release from a PLGA 50/50 microparticle was noticeably different to that of a γ-irradiated microparticle, encouraged us to determine if the un-irradiated and γ-irradiated PLGA 50/50 microparticles in the absence of hGH would degrade differently due to morphological changes. Therefore the final aim was to answer this question and help form fundamental links between the release characteristics of an important controlled release matrix and hydrolysis rates.

2. Experimental

2.1. Materials

PLGA RG502H (50:50 lactide:glycolide, I.V. 0.16–0.24, Bohringer-Ingleheim), PLGA RG752H (75:25 lactide:glycolide, I.V. 0.16–0.24, Bohringer-Ingleheim) PLA R202H (100:0 lactide:glycolide, I.V. 0.16–0.24, Bohringer-Ingleheim), pharmaceutical grade CO_2 (BOC Special Gasses) were used as received. Lactic acid (DL-Lactic acid, W261114, Sigma-Aldrich Company Ltd.) was dried at 70 °C for 1 day before use. Water used in the experiments was purified using a ELGA Purelab option-R water distiller (Up to 15 MΩ-cm, Type II water) and degassed using a Fisherbrand FB11004 ultrasonic bath at the relevant temperature (50 °C or 70 °C) for 15 min.

2.2. CriticalMix™ processing of PLGAs

CriticalMix™ is a novel one-step PGSS process used to produce microparticles with 100% encapsulation efficiency [30,32]. The method of PGSS, uses the ability of $scCO_2$ to depress the glass transition or melting temperature of polymers at ambient temperatures and moderate pressures. $scCO_2$ acts effectively as a molecular lubricant, thus liquefying polymers at temperatures significantly lower than those typically needed. This liquefication of the polymers allows APIs such as peptides and proteins that remain in the dry state during processing to be easily dispersed within the polymer matrix. CO_2 is the ideal choice for this application as it is readily available, non-toxic and interacts strongly with many biodegradable amorphous polyesters. In addition, the process is not drug specific and could provide a route to manufacturing many sustained release drug products across the pharmaceutical industry. The near ambient temperatures together with the absence of any aqueous or organic solvents makes the PGSS method particularly suited to the processing of thermally or solvent labile proteins and peptides, with the advantage that they can be encapsulated with no degradation or loss of activity. When suitable polymers such as PLGA, poly(ethylene glycol) or poloxamer and proteins such as bovine serum albumin or human growth hormone are exposed to $scCO_2$ in a pressure vessel, the polymer is liquefied, following mixing the mixture is depressurised through a nozzle whereby the CO_2 returns to a gaseous state by evaporation and the polymer solidifies. The batches used in this study were prepared