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Research paper

# Single processing step toward injectable sustained-release formulations of Triptorelin based on a novel degradable semi-solid polymer

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ABSTRACT

Poly(lactic acid) is a widely used polymer for parenteral sustained-release formulations. But its solid state at room-temperature complicates the formulation process, and elaborate formulation systems like microparticles and self-precipitating implants are required for administration. In contrast, hexylsubstituted poly(lactic acid) (hexPLA) is a viscous, biodegradable liquid, which can simply be mixed with the active compound. In this study, the feasibility to prepare injectable suspension formulations with peptides was addressed on the example of the GnRH-agonist Triptorelin. Two formulation procedures, of which one was a straight forward one-step cryo-milling-mixing process, were compared regarding the particle size of the peptide in the polymer matrix, distribution, and drug release. This beneficial method resulted in a homogeneous formulation with an average particle diameter of the incorporated Triptorelin of only 4.1 μm. The rheological behavior of the Triptorelin–hexPLA formulations was assessed and showed thixotropic and shear-thinning behavior. Viscosity and injectability were highly dependent on the drug loading, polymer molecular weight, and temperature. Nine formulations with drug loadings from 2.5% to 10% and hexPLA molecular weights between 1500 and 5000 g/mol were investigated in release experiments, and all displayed a long-term release for over 3 months. Formulations with hexPLA of 1500 g/mol showed a viscosity-dependent release and hexPLA–Triptorelin formulations of over 2500 g/mol a molecular weight-dependent release profile. In consequence, the burst release and rate of release were controllable by adapting the drug loading and the molecular weight of the hexPLA. The degradation characteristics of the hexPLA polymer during the *in vitro* release experiment were studied by following the molecular weight decrease and weight loss. Triptorelin–hexPLA formulations had interesting sustained-release characteristics justifying further investigations in the drug–polymer interactions and the *in vivo* behavior.


## 1. Introduction

Peptide pharmaceuticals are nowadays used in many therapeutic fields ranging from blood pressure control over osteoporosis treatment to cancer related hormone therapy. Their versatile structure, the facility to find new targets by directly using or altering native human peptides, and the possibility to chemically synthesize the product have resulted in more than 60 different peptides on the market [1]. Nevertheless, their poor oral bioavailability due to fast gastrointestinal degradation and insufficient absorption usually requires parenteral application [2]. Since most peptides have a short half-life time in the bloodstream, frequent injections are necessary to maintain therapeutic drug levels. This reduces a simple and convenient use of therapeutic peptides because most patients are reluctant to multiple injections [3]. Many strategies were utilized to reduce the number of injections by stabilizing the peptide, like prodrug concepts [4,5], the exchange of amino acids in the cleavage site [5,6], or chemical modification [5]. But only sustained-release formulations allow extending the injection interval to several weeks or even months. A class of active agents for which a treatment period of several months is desirable are the Gonadotropin-releasing hormone agonists (GnRH-agonists), like Leuprolide, Triptorelin, Goserelin, and Buserelin. The GnRH-agonists are extensively used in the treatment for long-lasting diseases like prostate cancer, endometriosis, *in vitro* fertilization, and precocious puberty, with the market size for the prostate cancer field alone exceeding 3 billion US dollars per year in 2003 [7].

Accordingly, a number of different sustained-release formulations for therapeutic peptides have been investigated and some

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entered the market, in large part based on implant- and microsphere technologies. Among these Viadur® was the only non-degradable implant, which needed to be removed after the treatment. It released Leuprolide over 1 year from a reservoir by osmotic control [8], but was recently withdrawn from the market, leaving only degradable sustained-release formulations. Zoladex® depot is a Goserelin containing implant based on poly(lactide-*co*-glycolide) (PLGA) [9], a similar technology as used in the Buserelin implant Profact®. Examples for the delivery of Leuprolide using microsphere technologies are the products Enantone®, Trenantone®, Sixantone®, and Lupron® depot [10]. Similar systems are used for Triptorelin sustained-release formulations as in Decapeptyl® N or Pamorelin® LA. Another possibility used in the Eligard® product is to dissolve the active substance together with the degradable polymer in a biocompatible solvent, which is diffusing after injection leading to the precipitation of an implant [11].

Although a large number of products are available, they all have certain inconveniences. For implants, the large needle size needed for administration causes discomfort and pain to the patient, as was reported for needles with a large diameter [12]. Besides the fact that microsphere formulations have to be resuspended prior to injection and that two-chamber devices are needed, the formulation processes are rather elaborated as summarized by Wischke and Schwendeman [13]. For the *in situ* forming implants, the use of *N*-methyl-2-pyrrolidone (NMP) and other organic solvents was reported to cause myotoxic effects and could result in damage to the injection site [14]. Furthermore, since all degradable sustained-release formulations for GnRH-agonists rely on PLGA, ageing of the polymer during storage might possibly lead to changes in the degradation characteristics and release [15]. As this is more probable to happen at elevated temperatures, most of the mentioned formulations have to be stored under cooled conditions.

To overcome these disadvantages, our aim was to investigate a directly injectable liquid formulation of a GnRH-agonist drug. Accordingly, an additional resuspension step and the use of organic solvents could be avoided. For this purpose, hexylsubstituted poly(lactic acid) (hexPLA) is an interesting excipient because it is liquid at room temperature and slowly degradable in the presence of water [16,17]. Like ordinary poly(lactic acid) (PLA), it has a polyester backbone and hence is subject to hydrolysis in an aqueous environment. In comparison with PLA, the longer aliphatic side chains act as internal plasticizers reducing the glass transition temperature, resulting in a viscous liquid. Furthermore, hexPLA of pharmaceutical grade can be prepared by a simple and environmentally friendly synthesis method and showed properties interesting for injectables like dry-heat sterilizability [18].

In this paper, we evaluated the potential of the polymer-excipient hexPLA to prepare sustained-release formulations with Triptorelin for a targeted release period of 3 months. Triptorelin as the active substance is reported to have better end point data for prostate cancer compared to other GnRH-agonists [19]. We established a UPLC method for the quantification of Triptorelin in buffer solution and in the formulation for the *in vitro* release experiments. Studies were carried out on the formulation process, the drug particle size, and the formulation rheology depending on the polymer molecular weight and drug loading. Finally, for different formulations, the degradation characteristics in buffer solution, as well as the *in vitro* release, were assessed.

## 2. Materials and methods

### 2.1. Materials

Triptorelin acetate was provided as a lyophilisate by Ferring GmbH (Kiel, Germany). 2-Hydroxyoctanoic acid, the monomer of hexPLA, was synthesized as previously published starting from heptaldehyde purchased from Sigma Aldrich (St. Louis, USA) [17]. Sulfuric acid 96% and trifluoroacetic acid were bought from Acros Organics (New Jersey, USA), and acetonitrile, tetrahydrofuran (THF), and isopropanol from VWR International (Leuven, Belgium). All starting materials and solvents were used as received.

### 2.2. Methods

#### 2.2.1. Polymer synthesis and sterilization

hexPLA polymers of a molecular weight of 1500 g/mol, 2500 g/mol, and 5000 g/mol were synthesized by the previously described polycondensation method [18]. In short, the monomer 2-hydroxyoctanoic acid was polymerized under heat and vacuum using 0.5% sulfuric acid as catalyst for the preparation of polymers with a molecular weight above 2000 g/mol. The polymer was purified after synthesis by precipitation from acetone into a cold aqueous solution of 0.1 M sodium bicarbonate followed by a filtration in acetone over Celite® 545 coarse. Remaining water and acetone was removed by evaporation. For polymers with a molecular weight below 2000 g/mol, the polycondensation was run catalyst free, rendering purification unnecessary. All polymers were sterilized using a European Pharmacopoeia standard dry-heat sterilization technique by heating them to 180 °C for 30 min.

#### 2.2.2. Formulation preparation and particle size determination

Two different methods were used to prepare Triptorelin–hexPLA formulations. In the two-step "separate milling and mixing method" the active compound was first micronized by milling for 5 min in a SPEX 6700 Freezer/Mill (SPEX Industries, Edison, USA). In the second step, the micronized powder was mixed with the viscous hexPLA polymer by kneading in plastic bags (Minigrip, Kennesaw, USA) until a homogeneous suspension formulation was obtained as verified by optical microscopy.

In the second method the lyophilized peptide and the polymer were cryo-milled together, which yielded a homogeneous distribution of the peptide in the polymer. This one-step "cryo-milling-mixing" method was run for 5 min. The hexPLA formulations were prepared containing 2.5%, 5.0%, and 10% (w/w) Triptorelin acetate. The molecular weight of the polymers was compared between before and after the cryo-milling process, using gel permeation chromatography (GPC) as described below. Furthermore, the particle size distribution in the formulations was analyzed using an Optiphot-2 microscope (Nikon, Tokyo, Japan) and ImageJ software (National Institutes of Health, Bethesda, USA).

#### 2.2.3. Rheology and injectability

At a constant temperature of 20 °C, the viscosity of the Triptorelin–hexPLA formulations was investigated at shear rates between 0.01 and 500 $s^{-1}$, delay time of 3 s, and integration time of 5 s. A Bohlin Instruments CVO 120 stress rheometer with a parallel plate set-up, type 20 mm (Bohlin Instruments, Cranbury, USA) and a gap of 1 mm was used. The dependence of temperature and viscosity was assessed at a shear rate of 0.1 $s^{-1}$ and in a temperature range between 15 °C and 37 °C at measurement intervals of 1 °C.

The injectability was exemplary tested with a formulation containing 10% Triptorelin acetate in hexPLA of 2500 g/mol. The formulation was filled into a silanized glass syringe with an inner diameter of 6.3 mm connected to a 21 G and 23 G needle, respectively, of 25 mm length. The ejection speed was fixed at 10 mm/min, and the necessary force to empty the syringe was measured on a dynamometer HBM Load Type 23H2 (Hottinger Baldwin Messtechnik, Volketswil, Switzerland) fixed in press type RM 50 (Schenck AG, Nänikon, Switzerland).