Innovative platform technologies for stabilization and controlled release of proteins from polymer depots
Stankovic, Milica

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Tailored protein release from biodegradable poly(ε-caprolactone-PEG)-b-poly(ε-caprolactone) multiblock-copolymer implants

Milica Stanković a, Jasmine Tomar a, Christine Hiemstra b, Rob Steendam b, Henderik W. Frijlink a, Wouter L.J. Hinrichs a

a Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, A. Deusinglaan 1, 9713 AV, Groningen, The Netherlands
b InnoCore Pharmaceuticals, L.J. Zielstraweg 1, 9713 GX, Groningen, The Netherlands

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Chapter 5

Abstract

In this study, the *in-vitro* release of proteins from novel, biodegradable phase-separated poly(ε-caprolactone)-PEG)-*b*-poly(ε-caprolactone), [PCL-PEG]-*b*-[PCL]) multiblock copolymers with different block ratios and with a low melting temperature (49 - 55 °C) was studied. The effect of block ratio and PEG content of the polymers (i.e. 22.5, 37.5 and 52.5 wt%) as well as the effect of protein molecular weight (1.2, 5.8, 14, 29 and 66 kDa being goserelin, insulin, lysozyme, carbonic anhydrase and albumin, respectively) on protein release was investigated. Proteins were spray-dried with inulin as stabilizer to obtain a powder of uniform particle size. Spray-dried inulin-stabilized proteins were incorporated into polymeric implants by hot melt extrusion. All incorporated proteins fully preserved their structural integrity as determined after extraction of these proteins from the polymeric implants. In general, it was found that the release rate of the protein increased with decreasing molecular weight of the protein and with increasing the PEG content of the polymer. Swelling and degradation rate of the copolymer increased with increasing PEG content. Hence, release of proteins of various molecular weights from [PCL-PEG]-*b*-[PCL] multi-block copolymers can be tailored by varying the PEG content of the polymer.
1. Introduction

The use of proteins and peptides as therapeutic agents has significantly increased in the last few decades. Since the 1980’s more than 200 biopharmaceuticals have been registered [1]. However, despite extensive research, the development of patient-friendly dosage forms capable of maintaining the concentration of the proteins in plasma at pharmacologically effective levels for extended periods of time is still a major challenge [2]. Oral delivery of proteins and peptides has so far been unsuccessful, since it results in a low bioavailability due to degradation caused by gastrointestinal enzymes and low permeability through the gastrointestinal membrane. Therapeutic proteins are preferably administered parenterally as an aqueous solution, but due to their short half-life most proteins require frequent injection. For these reasons, research on the development of parenteral sustained release (or depot) formulations has expanded enormously during the last few decades. Biodegradable polymers such as poly(DL-lactide-co-glycolide) (PLGA) or poly(DL-lactide) (PDLA) have been widely applied as release controlling polymers in microparticle and implant-based depot formulations for peptides and proteins [2–4]. These polymers degrade in the body due to hydrolysis and are finally metabolized into water and carbon dioxide [5]. Sustained release depot formulations are typically administered parenterally via subcutaneous or intra-muscular injection, with the use of customized applicators [6,7]. Due to incompatibility of both the polymers and the drug molecules with standard sterilization procedures such as heat, autoclavation or radiation, these depot formulations are usually manufactured aseptically.

Microparticle-based sustained release depot formulations are typically manufactured via process routes that require the use of organic solvents to dissolve the polymers and aqueous media to extract the organic solvents from the microparticles. It is well known that the resulting organic solvent - water interfaces can have a devastating effect on the structural integrity and bioactivity of protein therapeutics. Processes such as molding, melt compression and hot melt extrusion offer the possibility for solvent free processing, which make them attractive alternatives for the manufacturing of sustained release depot formulations for proteins. However, these production processes often expose the proteins to heat and shear stresses. Hot melt extrusion of polymers like PLGA and PDLA requires high processing temperatures, which in combination with the shear stresses may lead to protein degradation [8].

In a previous study, we have synthesized novel hydrophilic multiblock copolymers composed of semi-crystalline poly(ε-caprolactone) (PCL) blocks and amorphous blocks consisting of PCL and poly(ethylene glycol) (PEG). We have shown that the combination of this type of hydrophilic [PCL-PEG]-b-[PCL] polymers
and low temperature HME allows incorporation of proteins into the implants without protein degradation and controlled release of fully intact protein [9].

When formulating proteins and peptides into polymeric implants, one needs to take into account the various factors that affect the release kinetics. Protein release from polymeric matrices is governed by the physico-chemical properties of both polymer and drug, as well as by the conditions at the site of release. Among others, protein release is affected by protein charge [10], protein loading [9] and protein molecular weight [11]. Essential polymer properties include the degradation rate and swellability [12], which depend on composition and molecular weight of the polymer [13]. Although several studies have already been conducted on the importance of protein molecular weight on the release from polymeric depots [14–16], the aim of this study was to investigate in more detail the correlation between protein molecular weight and protein release from this new type of hydrophilic [PCL-PEG]-b-[PCL] polymers with different PEG contents. We synthesized [PCL-PEG]-b-[PCL] multiblock co-polymers with different [PCL-PEG] / [PCL] ratios and prepared protein-loaded implants thereof by HME. The release of five proteins with different molecular weight, i.e. goserelin (1.2 kDa), recombinant human insulin (5.8 kDa), lysozyme (14 kDa), carbonic anhydrase (29 kDa) and bovine serum albumin (66 kDa) from [PCL-PEG]-b-[PCL] polymers with varying PEG content was evaluated. Additionally, the degradation behavior of the polymers was studied to obtain more insight into the release mechanism of the proteins from these polymeric implants.

2. Materials and methods

2.1. Materials

Goserelin acetate (Gos) was purchased from BCN (Barcelona, Spain). Lyophilized human recombinant insulin (Ins) (26.9 units/mg, ~5.8 kDa), lyophilized Lys (Lys) from chicken egg white (70,000 units/mg, ~14 kDa), lyophilized carbonic anhydrase (CA) from bovine erythrocytes (protein >3500 W-A units/mg, ~29 kDa) and lyophilized albumin from bovine serum (BSA), (protein >96 %, ~66 kDa), Micrococcus Lysodeikticus, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride and trifluoroacetic acid (TFA) were all purchased from Sigma (St.Louis, Missouri, USA). Inulin 4000 g/mol was a gift from Sensus (Rosendaal, The Netherlands). Sodium azide, ε-caprolactone, 1,4-butanediol and 1,4-dioxane were purchased from Acros Organics (Geel, Belgium). Acetonitrile (HPLC gradient grade) was purchased from Biosolve® (Valkenswaard, The Netherlands). Hydrochloric acid
Tailored protein release from biodegradable poly(ε-caprolactone-PEG)-
b-poly(ε-caprolactone) multiblock-copolymer implants

37 % (reagent grade) was purchased from VWR International Ltd. (Leicestershire, UK). Tri (hydroxymethyl) aminomethane was purchased from Merck (Darmstadt, Germany). Stannous octoate was obtained from Sigma. 1,4-Butanediisocyanate was purchased from Bayer. Deuterated chloroform and lithium bromide were obtained from Fisher; dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and PEG standards (FLUKA, Buchs, Switzerland) were purchased from Sigma.

2. 2. Polymer synthesis

Low molecular weight poly(ε-caprolactone) (PCL) (Mw 4000 g/mol) and poly(ε-caprolactone)-PEG<sub>1500</sub>-poly(ε-caprolactone) (PCL-PEG<sub>1500</sub>) (Mw 2000 g/mol) prepolymer were synthesized by standard stannous octoate catalyzed ring-opening polymerization, as described previously [9]. Shortly, ε-caprolactone was dried over CaH₂ and distilled under reduced pressure in a nitrogen atmosphere prior to polymerization. PEG with a molecular weight of 1500 g/mol (PEG<sub>1500</sub>) was dried for 17 h at 90 °C under vacuum. 1,4-Butanediol and 1,4-butanediisocyanate were distilled under reduced pressure. The purity of distilled ε-caprolactone, 1,4-butanediol and 1,4-butanediisocyanate was confirmed by ¹H NMR (CDCl₃). PCL and PCL-PEG<sub>1500</sub> prepolymer were then chain-extended with 1,4-butanediisocyanate to prepare [poly(ε-caprolactone)-PEG<sub>1500</sub>-poly(ε-caprolactone)]<sub>2000</sub>-b-[poly(ε-caprolactone)]<sub>4000</sub> [PCL-PEG<sub>1500</sub>]<sub>b</sub>-[PCL] multiblock co-polymers with different [PCL-PEG<sub>1500</sub>]/[PCL] block ratios (30/70, 50/50 and 70/30 w/w abbreviated as 30[PCL-PEG<sub>1500</sub>]-70[PCL], 50[PCL-PEG<sub>1500</sub>]-50[PCL] and 70[PCL-PEG<sub>1500</sub>]-30[PCL]) as described in [9]. Shortly, the prepolymer PCL and PCL-PEG<sub>1500</sub> were introduced into a three-necked bottle under nitrogen atmosphere. Dry 1,4-dioxane (distilled over sodium wire) was added to a polymer concentration of 30 wt% and the mixture was heated to 80°C to dissolve the prepolymer. 1,4-Butanediisocyanate (BDI) was added and the reaction mixture was stirred mechanically overnight for 20 h. After cooling to room temperature, the reaction mixture was transferred into a tray, frozen and vacuum-dried at 30 °C to remove 1,4-dioxane. The residual 1,4-dioxane content, as measured by GC headspace, was less than 200 ppm for all three multiblock co-polymers, showing effective removal of 1,4-dioxane by vacuum drying.
2.3. Polymer characterization

$^1$H NMR was used to verify the overall ε-caprolactate / PEG (CL/PEG) monomer ratio of the multiblock co-polymers after synthesis and during degradation. $^1$H NMR was performed on a VXR Unity Plus NMR spectrometer (Varian, California, USA) operating at 300 MHz. The $d_1$ waiting time was set to 20 s, and the number of scans was 16-32. $^1$H NMR samples were prepared by dissolving 10 mg of multiblock co-polymer into 1 mL of deuterated chloroform (CDCl$_3$), and the spectrum was determined from 0 - 8 ppm using CHCl$_3$ present as trace element in CDCl$_3$ as reference. The CL/PEG molar ratio was calculated from the O-CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C (O)- methylene group of PCL at $\delta$ 2.2-2.4 and the –CH$_2$CH$_2$-O methylene groups of PEG at $\delta$ 3.6-3.7.

The intrinsic viscosity of multiblock co-polymers dissolved in chloroform was determined by measuring at three different polymer concentrations at a temperature of 25 °C using an Ubbelohde viscometer (DIN, type 0C Schott Geräte supplied with a Schott AVS-450 Viscometer equipped with a water bath).

The residual 1,4-dioxane content of the multiblock copolymers was determined using a GC-FID headspace method. Measurements were performed on a GC-FID Combi Sampler supplied with an Agilent Column, DB-624 / 30 m / 0.53 mm. Samples were prepared in DMSO. 1,4-Dioxane content was determined using 1,4-dioxane calibration standards.

The molecular weight of the multiblock co-polymers after synthesis and during degradation was determined using size exclusion chromatography coupled to refractive index detector (SEC-HPLC, Waters, Breeze, USA). The sample (0.01 g) was dissolved in DMF (1 mL). PEG standards having molecular weights of 1 - 218 kg/mol were prepared likewise. Samples and PEG standards were injected (50μL) onto the SEC column (Thermo Fisher, Column 1: Plgel 5 μm 500 Å, column 2: Plgel 5 μm 500 Å, column 3: Plgel 5 μm 104 Å, eluent: DMF with 0.1 M LiBr, flow: 1 mL/min). Polymers were detected by refractive index. The apparent molecular weights were then calculated with the aid of the PEG standards calibration curve.

Thermal characteristics of the multiblock co-polymers were measured by modulated differential scanning calorimetry (DSC) using a Q2000 differential scanning calorimeter (TA Instruments, Ghent, Belgium). Samples of 5 - 10 mg were heated from –85 °C to 100 °C at a rate of 2 °C/min, with 0.318 °C amplitude over a 60 s period. During the measurement, the sample cell was purged with nitrogen gas. The reversed heat flow was used to determine the glass transition temperature (midpoint of the transition, determined via inflection), and the total heat flow was used to determine the melting temperature (maximum of endothermic peak). The heat of fusion was calculated from the surface area of the melting endotherm.
2. 4. Spray-drying

Spray-drying was performed using a B-290 mini spray drier (Büchi Labortechnik AG, Flawil, Switzerland). Proteins (2.5 mg/ml) were separately dissolved in demineralized water and an aqueous solution of inulin (25 mg/ml) was added, to obtain a protein/inulin weight ratio of 1/10. Samples were spray-dried under the following conditions: atomizing pressure of 55 psi, liquid feed rate of 2.5 mL/min, an inlet temperature of 100 °C and the outlet temperature was determined to be 58 °C ± 3 °C. The obtained spray-dried powder was stored in a desiccator overnight until further use.

2. 5. Particle size measurements

The particle size distribution of the spray-dried samples was determined by laser diffraction. Small amounts of material (20 – 30 mg) were dispersed using a RODOS dispersing system at 3.0 bars. The particle size distribution was determined with a KA laser diffraction apparatus (Sympatec GMbH, Clausthal-Zellerfeld, Germany) equipped with a 175 mm lens. Calculations were based on Fraunhofer theory.

2. 6. Hot melt extrusion (HME)

HME was performed using a HAAKE MiniLab Rheomex CTW5 (Thermo-Electron) co-rotating twin-screw extruder equipped with conveyer belt to stretch the molten material. The extrusion process was performed with a screw speed of 10 - 20 rpm, developing a torque of 10 - 20 Nm. In total, ten formulations were prepared (Table 1). Powder blends (5.5 g) containing protein / inulin spray-dried particles and polymer at a weight ratio of 11/89 were manually mixed using a mortar and pestle and then fed into the preheated barrel of the extruder. The temperature used for extrusion was 55 °C for 30[PCL-PEGAsian 1500]-70[PCL] and 49 °C for 50[PCL-PEGAsian 1500]-50[PCL] and 70[PCL-PEGAsian 1500]-30[PCL]. A cylindrical die of 0.5 mm was used resulting in strands with a diameter of 0.35 mm (± 0.05), as measured with an in-line laser. Strands were cut into 1 cm x 0.35 mm pieces and stored in a freezer at -20°C until further evaluation. Polymer only implants (used for the degradation study) were prepared similarly by feeding the preheated extruder with polymer without any further additives.
Table 1. Implant formulations prepared by HME. Protein/inulin weight ratio is 1/10. Protein/inulin loading is 11 wt%.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>30[PCL-PEG(_{1500})]-70[PCL] (22.5 wt% PEG)</th>
<th>50[PCL-PEG(_{1500})]-50[PCL] (37.5 wt% PEG)</th>
<th>70[PCL-PEG(_{1500})]-30[PCL] (52.5 wt% PEG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight of proteins</td>
<td>1.2 kDa Gos/Inulin(<em>{30})[PCL-PEG(</em>{1500})]-70[PCL]</td>
<td>Gos/Inulin(<em>{50})[PCL-PEG(</em>{1500})]-50[PCL]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.8 kDa Ins/Inulin(<em>{30})[PCL-PEG(</em>{1500})]-70[PCL]</td>
<td>Ins/Inulin(<em>{50})[PCL-PEG(</em>{1500})]-50[PCL]</td>
<td>Lys/Inulin(<em>{70})[PCL-PEG(</em>{1500})]-30[PCL]</td>
</tr>
<tr>
<td></td>
<td>14 kDa Lys/Inulin(<em>{30})[PCL-PEG(</em>{1500})]-70[PCL]</td>
<td>Lys/Inulin(<em>{50})[PCL-PEG(</em>{1500})]-50[PCL]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29 kDa CA/Inulin(<em>{50})[PCL-PEG(</em>{1500})]-50[PCL]</td>
<td>BSA/Inulin(<em>{50})[PCL-PEG(</em>{1500})]-50[PCL]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 kDa BSA/Inulin(<em>{50})[PCL-PEG(</em>{1500})]-50[PCL]</td>
<td>BSA/Inulin(<em>{70})[PCL-PEG(</em>{1500})]-30[PCL]</td>
<td></td>
</tr>
</tbody>
</table>

2. 7. Polymer mass loss and water content during degradation

The degradation of polymeric implants without incorporated proteins (prepared as described in section 2.6) was studied to obtain more insight into the role of polymer degradation in the release mechanism. About 130 mg of polymeric implants (n = 3) was placed in plastic vials and 25 mL of 100 mM phosphate buffer (pH 7.4, 100 mM, 9.1 mM NaCl, 0.02 wt% NaN\(_3\)) was added to each vial. The vials were then incubated in an oven at 37 ºC. At various time intervals, samples were removed from the buffer and washed with ultra pure water over 0.45 μm filters to remove the buffer salts. Adherent water was removed from the implants with a tissue where after their wet mass (\(m_{\text{wet},t}\)) was determined. Samples were then dried in a desiccator and additionally in a vacuum oven for 24 h until constant mass (\(m_{\text{dry},t}\)). Water content was measured after the implants had reached an equilibrium value (after 1 day); water content and remaining mass were determined using Eq. (1) and (2), respectively.

\[
\text{Water content} \ (\%) = 100 \times \frac{m_{\text{wet},t} - m_{\text{dry},t}}{m_{\text{wet},t}} \quad (1)
\]

\[
\text{Mass loss} \ (\%) = 100 \times \frac{m_{\text{dry},0} - m_{\text{dry},t}}{m_{\text{dry},0}} \quad (2)
\]

Where \(m_{\text{dry},0}\) and \(m_{\text{dry},t}\) are the masses of the dry sample at day 0 and dry sample at day \(t\).

\text{In-vitro} degradation of the 30[PCL-PEG\(_{1500}\)]-70[PCL] and 50[PCL-PEG\(_{1500}\)]-50[PCL] polymers was studied for 140 days. However, the 70[PCL-PEG\(_{1500}\)]-30[PCL] polymer was assessed for its degradation characteristics for only 7 days, since partial dissolution of the polymer occurred after that period of time.
2.8. Analysis of protein content

To determine the actual protein content and structural integrity of protein after extrusion, proteins were extracted from the implants. Samples randomly taken from the extrusion run were weighed and to each of the samples 1.5 ml of acetonitrile was added to dissolve the polymer. After the polymer was fully dissolved, the sample was centrifuged (Microcentrifuge SIGMA 1-14, Shropshire, United Kingdom) and the supernatant consisting of dissolved polymer was removed. The procedure was repeated three times and the remaining protein pellet was dried in a desiccator overnight to remove residual solvent. The protein pellet was dissolved in 100 mM phosphate buffer (pH 7.4, 9.1 mM NaCl, 0.02 wt% NaN₃) and analyzed as described in section 2.9. Control experiments using physical mixtures of polymer and protein showed that the extraction procedure did not affect the biological activity of the protein and that the protein could be fully recovered and remained structurally intact by the extraction procedure.

2.9. In-vitro release testing

Protein containing implants (70 - 90 mg, n = 3) were weighed into glass tubes, and 1.5 mL of 100 mM phosphate buffer (pH 7.4, 9.1 mM NaCl, 0.02 wt% NaN₃) was added. Thereafter, the tubes were capped and placed in a shaking water bath (GFL, model 1083, Burgwerdel, Germany) thermostated at 37°C, and incubated for a total of 160 days. After various time intervals, 1.25 mL aliquots were removed for HPLC analysis, as described in section 2.10, and refreshed. The cumulative amount of released protein within 4h was considered as a burst release.

To elucidate the mechanisms of release of the proteins from the three different polymers, release data were plotted into Korsmeyer-Peppas’ model (Eq. (3)).

\[
\frac{Q_t}{Q_0} = K_p \times t^n
\]

where \(Q_t\) is amount of drug released in time \(t\), \(Q_0\) is initial amount of the drug in the implant, \(t\) is time, \(n\) is diffusional exponent indicative of the transport mechanism and \(K_p\) is Korsmeyer-Peppas constant incorporating structural and geometric characteristics of the dosage form.

Using Peppas’ model, the diffusional exponent \(n\) was calculated from the fitted linear regression lines of log percent of drug released versus log time (\(n\) was equal to the slope taken for the first 60 % of drug released). When \(n \leq 0.45\) for a cylindrically shaped release system, then the drug is released by Fickian diffusion. For \(n\) values between 0.45 and 0.89, non-Fickian or anomalous transport is the predominant mechanism of release, where both drug diffusion and polymer swelling and erosion
play a role. For \( n = 0.89 \) (Case II transport) release does not depend on time and it corresponds to zero order kinetics. Finally, values of \( n > 0.89 \) are indicative for a release mechanism known as super case II transport where the release is governed by high polymer swelling and thus diffusion of the drug [17–19]. Coefficients of determination (\( R^2 \)) were calculated for all formulations as a measure of linearity of data sets.

Further, using a mathematical model that describes the similarity factor \( f_2 \), two similar data sets can be compared (Eq. (4)). The similarity factor \( f_2 \) equals 100 when two data sets are equivalent, while 10 % of difference between sets would give an \( f_2 \) value of 50. Therefore, it is accepted that two data sets are highly similar or equivalent if the \( f_2 \) value is between 50 and 100, while different if the \( f_2 < 50 \) [18].

\[
f_2 = \log \left\{ \left[ 1 + \left( \frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}
\]  (4)

2. 10. Protein quantification measurements

The concentration of the proteins Lys, CA and BSA in aqueous solutions was determined by RP-HPLC using a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Sunnyvale, CA, California), protein detection at 280 nm and a C18 ProZap LC/MS reversed phase column (Grace Davidson, Deerfield, IL, USA) (20 mm x 4.6 mm, 1.5 µm). The composition of the mobile phase was 0.1 vol% TFA in acetonitrile (eluent A) and 0.1 vol% TFA in millipore water (eluent B). The quantification of the proteins was carried out in gradient elution mode using the following scheme: 0 min - 1 min 30 vol% A, 70 vol% B; 1 – 3 min 60 vol% A – 40 vol% B; 3.01 - 6 min 30 vol% A, 70 vol% B. The retention time of Lys, BSA and CA was 1.19 min, 1.29 min and 1.57 min, respectively. The concentration of Gos was determined by RP-HPLC using a Dionex Ultimate 3000 HPLC system (Thermo Scientific, Sunnyvale, CA, USA). Waters C18 reverse phase column (4.6 mm x 150 mm, 5 µm) (Milford, MA, USA) was used in isocratic mode using 0.1 vol% TFA in 76/24 v/v water / acetonitrile as eluent. The concentration Gos was determined spectrophotometrically at a wavelength of 214 nm. Ins was quantified using a UV-VIS method, with Unicam UV500 spectrophotometer (Madison, USA). Absorbance of Ins was recorded at wavelength of 226 nm using a quartz cuvette (L=10 mm).

2. 11. Structural integrity of the proteins

Since the various proteins have a different sensitivity for temperature (and shear stress), it is important to determine their structural integrity after spray-drying and HME. The integrity of Gos was evaluated using the RP-HPLC method as described
The assay is based on the time taken by saturated CO$_2$ solution to drop the pH of a 0.02 M Tris-HCl buffer from 8.3 to 6.3 at 0 ºC. The total activity ($A_{tot}$) of CA was calculated using Eq. (5). A calibration curve was established using aqueous solutions of CA of various concentrations. The retained activity ($A_{ret}$) of CA after spray-drying and HME was determined using Eq. (6).

$$A_{tot} = \frac{t_0 - t}{t}$$

Where $t$ and $t_0$ are the times recorded with and without enzyme, respectively.

$$A_{ret} = \frac{C_A}{C_0} \times 100\%$$

Where $C_A$ is the active concentration of extracted CA from samples after HME and $C_0$ is the concentration of the CA control.

Since no biological assay for BSA is available, its integrity after processing was studied using the RP-HPLC method as described in section 2.10 (determination of additional peaks) and SDS-PAGE as described by Laemmli [23] to evaluate whether aggregation occurred. Electrophoresis was performed at a constant current mode of 100 volts and gels were stained with Coomassie brilliant blue.

### 3. Results

#### 3.1. Polymer characterization

$^1$H NMR analysis confirmed that the CL/PEG molar ratios of the [PCL-PEG]-b-[PCL] multi-block copolymers were in line with the in weight values (Table 2) indicating that the polymer compositions were according to the targets. DSC thermograms confirmed the phase-separated nature of the multiblock copolymers, showing a glass transition temperature ($T_g$) between -60 and -56 ºC (Figure 1b), which can be attributed to phase-mixed amorphous PEG and PCL.
and 50[PCL-PEG\textsubscript{1500}]-50[PCL] exhibited two melting endotherms, which can be attributed to melting of PEG crystals at 13 °C and 40 °C and to melting of PCL crystals at 50 – 56 °C (Figure 1a). For 70[PCL-PEG\textsubscript{1500}]-30[PCL] polymer, however, three instead of two melting endotherms were observed. The melting peaks at 25 °C and 40 °C can be attributed to melting of two crystalline modifications of PEG as described previously [24]. The third melting endotherm at 60 °C can be ascribed to melting of PCL crystals. The crystallinity of the PCL block was calculated to be 58 %, 70 % and 50 % for 30[PCL-PEG\textsubscript{1500}]-70[PCL], 50[PCL-PEG\textsubscript{1500}]-50[PCL] and 70[PCL-PEG\textsubscript{1500}]-30[PCL], respectively, assuming a heat of fusion (ΔH) of 139.9 J/g for 100 % crystalline PCL [25]. The crystallinity of the PEG domains was 16 %, 23 %, 41 % for 30[PCL-PEG\textsubscript{1500}]-70[PCL], 50[PCL-PEG\textsubscript{1500}]-50[PCL] and 70[PCL-PEG\textsubscript{1500}]-30[PCL], respectively, assuming a ΔH of 165.5 J/g for 100 % crystalline PEG [26]. The melting temperature of PEG (Tm) increased with increase in [PCL-PEG\textsubscript{1500}] / [PCL] block ratio. We propose that this increase in Tm is due to the formation of larger and more perfect PEG crystals at higher PEG content [24], [25]. This is supported by the increasing melting enthalpy of the PEG crystals from 14 J/g for 30[PCL-PEG\textsubscript{1500}]-70[PCL] to 53 J/g for 70[PCL-PEG\textsubscript{1500}]-30[PCL]. The intrinsic viscosity and the molecular weight of the multiblock copolymers, as determined by SEC, are shown in Table 2. SEC was performed relative to PEG standards and thus apparent molecular weight values were obtained. Number averaged molecular weight of the multiblock copolymers was in the range of 26 x 10\textsuperscript{3}; 28 x 10\textsuperscript{3} and 13 x 10\textsuperscript{3} g/mol for 30[PCL-PEG\textsubscript{1500}]-70[PCL], 50[PCL-PEG\textsubscript{1500}]-50[PCL] and 70[PCL-PEG\textsubscript{1500}]-30[PCL], respectively, while PCL-PEG\textsubscript{1500} and PCL prepolymers have molecular weight of 2000 and 4000 g/mol, respectively, again confirming the successful chain-extension of the prepolymers.

**Figure 1. Thermograms of multiblock copolymers;**

- a) total heat flow -40 to 100 °C showing the melting endotherm of PEG and PCL;
- b) reversing heat flow showing the glass transition of PCL

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Table 2. Characterization of the multiblock-copolymers. *Determined after HME.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>CL/PEG molar ratio (-)</th>
<th>IV (dL/g)</th>
<th>Tg (°C)</th>
<th>H (J/g)</th>
<th>Tm (°C)</th>
<th>H NMR</th>
<th>Mn (g/mol)</th>
<th>Mw (g/mol)</th>
<th>PDI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>30[PCL-PEG(_{1500})-70[PCL]</td>
<td>0.84</td>
<td>45</td>
<td>-60</td>
<td>13</td>
<td>44.2</td>
<td>44.2</td>
<td>25,664</td>
<td>48,287</td>
<td>1.88</td>
</tr>
<tr>
<td>50[PCL-PEG(_{1500})-50[PCL]</td>
<td>0.75</td>
<td>19.6</td>
<td>-56</td>
<td>21</td>
<td>21.3</td>
<td>21.3</td>
<td>28,464</td>
<td>54,233</td>
<td>1.79</td>
</tr>
<tr>
<td>70[PCL-PEG(_{1500})-30[PCL]</td>
<td>0.41</td>
<td>10.1</td>
<td>-59</td>
<td>30</td>
<td>11.1</td>
<td>11.1</td>
<td>12,965</td>
<td>22,316</td>
<td>1.80</td>
</tr>
</tbody>
</table>
3.2. Polymer degradation

The mass loss after 140 days of polymer-only implants with low (30[PCL-PEG\textsubscript{1500}]-70[PCL], 22.5 %) and intermediate PEG content (50[PCL-PEG\textsubscript{1500}]-50[PCL], 37.5 %) was 8 % and 15 %, respectively (Figure 2). The polymer with the highest PEG content (70[PCL-PEG\textsubscript{1500}]-30[PCL], 52.5 % PEG) lost around 52 % of its mass already within one day and only marginal additional mass loss occurred within the next 6 days. Unfortunately, handling of this polymer was difficult due to its high swellability. Therefore, and because all incorporated protein was released from this polymer already within 7 days, the degradation study of this polymer was terminated after 7 days.

With increasing amounts of PEG incorporated in the polymer, the water content slightly increased, i.e. 56 % for 30[PCL-PEG\textsubscript{1500}]-70[PCL], 60 % for 50[PCL-PEG\textsubscript{1500}]-50[PCL], to 78 % for 70[PCL-PEG\textsubscript{1500}]-30[PCL], after 1 day of incubation. Water content remained constant during 140 days.

SEC was done to determine molecular weight decrease of polymer during degradation. It was observed that polymer 30[PCL-PEG\textsubscript{1500}]-70[PCL] showed only minor molecular weight decrease, while for 50[PCL-PEG\textsubscript{1500}]-50[PCL] molecular weight decrease started gradually and proceeded to 58 % of its original value in 140 days. Polymer 70[PCL-PEG\textsubscript{1500}]-30[PCL] showed a small increase in average molecular weight during 7 days. The polydispersity index ($M_w / M_n$) remained around 1.8 for all samples during degradation as shown in Table 2.

![Figure 2. Mass loss (%), water uptake (%) and molecular weight loss (%) of multiblock co-polymers 30[PCL-PEG\textsubscript{1500}]-70[PCL] (○), 50[PCL-PEG\textsubscript{1500}]-50[PCL] (■) and 70[PCL-PEG\textsubscript{1500}]-30[PCL] (▲) during degradation.]

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3. 3. Spray-drying, HME and determination of protein content

Spray-drying of protein / inulin solutions resulted in all cases in a powder with an average particle size of around 2.5 μm, while span values indicated narrow size distributions within the batch (results not shown). Further, as expected, HME of [PCL-PEG]-b-[PCL] polymers could be performed at temperatures between 49 and 55 °C. Extraction of the proteins from different parts of the polymeric implants showed uniform distribution of the protein / inulin powders within the polymeric matrices as indicated by the small standard deviation, with actual loading varying only slightly from the theoretical loading (1 %) as shown in Table S1 (supporting information). The slightly lower loading of insulin found after extraction was attributed to less effective extraction of insulin from the implants.

3. 4. Protein stability

The integrity of the proteins was studied after spray-drying and HME (n = 3). Gos and Ins were analyzed by RP-HPLC, because degradation of both substances can be observed by the presence of additional peaks in chromatograms. For both Gos and Ins extracted from different polymers, 30[PCL-PEG\(_{1500}\)]-70[PCL] and 50[PCL-PEG\(_{1500}\)]-50[PCL], no additional peaks were observed as compared to the control (data not shown). The stability of Lys and CA was studied by both RP-HPLC and bioactivity assays. Also in these cases no additional peaks in RP-HPLC chromatograms were observed (data not shown). The activity of Lys was found to be 100 ± 4.7 and 104 ± 8.1 % for Lys extracted from the 30[PCL-PEG\(_{1500}\)]-70[PCL] and 50[PCL-PEG\(_{1500}\)]-50[PCL] polymer, respectively. Regarding CA, measured activity of protein extracted from the 50[PCL-PEG\(_{1500}\)]-50[PCL] polymer was found to be 99 ± 1.4 %, indicating no loss in activity after spray-drying and HME. The integrity of extracted BSA was investigated using SDS-PAGE. As shown in Figure S1 (supporting information), a single band corresponding to the molecular weight of BSA, i.e. 66 kDa, was found, which indicates preservation of the integrity of BSA after spray-drying and HME with both 50[PCL-PEG\(_{1500}\)]-50[PCL] and 70[PCL-PEG\(_{1500}\)]-30[PCL].

3. 5. In-vitro protein release

*In-vitro* release of the proteins from the [PCL-PEG]-b-[PCL] implants was studied during 160 days. Polymer 30[PCL-PEG\(_{1500}\)]-70[PCL] (with 22.5 wt% PEG) released 59 % of Gos in the first 4h, after which a slightly slower release of Gos was noticed which accumulated to 100 % within 8 days. Release of Ins from 30[PCL-PEG\(_{1500}\)]-70[PCL] was slower and more continuous with 8 % of burst and release completed.
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within 80 days. Finally, release of Lys from this polymer was slowest, with 13 % burst and continuous release of 90 % of protein within 160 days (Figure 3a).

Polymer 50[PCL-PEG$_{1500}$]-50[PCL] (with intermediate PEG content, 37.5 wt%) also showed an increased release rate when the molecular weight of the protein was decreased (Figure 3b). The fastest release was observed for Gos (burst release 53 %, complete release in 8 days), followed by biphasic release of Ins (burst release 12 %, complete release in 80 days), continuous release of Lys (4 % burst, 100 % released in 160 days) and biphasic release of CA (the lag phase followed by the release of 80 % of protein in 160 days). Additionally, only around 3 % of release was noticed for the largest protein, i.e. BSA. The polymer with the highest PEG content (52.5 wt%), 70[PCL-PEG$_{1500}$]-30[PCL], released more than 60 % of Lys in the first 4 hours. The remaining protein was released within the next 7 days. Also, rapid release was observed for BSA, i.e. 40 % of protein was released after 4h and all protein was released within 7 days (Figure 3c). Because the release profiles of Gos as well as Ins from implants prepared from either 30[PCL-PEG$_{1500}$]-70[PCL] or 50[PCL-PEG$_{1500}$]-50[PCL] looked quite similar, the release data were inserted into the $f_2$ equation. It was found that, for Gos, $f_2$ was 72, indicating high similarity or equivalence between release of Gos from both polymers. $f_2$ value for Ins was 55, showing similarity as well (but to a lower extent) between the two release profiles.

Figure 3. Release profiles of proteins from a) 30[PCL-PEG$_{1500}$]-70[PCL], b) 50[PCL-PEG$_{1500}$]-50[PCL] and c) 70[PCL-PEG$_{1500}$]-30[PCL].

Gos (■), Ins (■), LYS (●), CA (▲), BSA (×).
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To obtain a better understanding of the mechanisms of release from the different formulations, all release data were fitted into Korsmeyer-Peppas model (Table 3). Except for the release date of Gos from 30[PCL-PEG\textsubscript{1500}]-70[PCL], the relatively high \( R^2 \) of 0.934 – 0.957 for Gos for 50[PCL-PEG\textsubscript{1500}]-50[PCL] and Ins from both 30[PCL-PEG\textsubscript{1500}]-70[PCL] and 50[PCL-PEG\textsubscript{1500}]-50[PCL] indicate a good fit into this model. The \( n \) value was lower than 0.45, which implies that the release was diffusion controlled. The release of Lys from both polymers showed lower values for \( R^2 \) of 0.913 and 0.861, while an \( n \) value of 0.239 for 30[PCL-PEG\textsubscript{1500}]-70[PCL] and 0.474 for 50[PCL-PEG\textsubscript{1500}]-50[PCL], suggested diffusion based release and anomalous transport, respectively. CA, having a lag phase of 45 days, showed a poor fit into the model. The diffusional exponent \( n = 0.597 \) indicated anomalous transport of this protein through the polymer matrix. From 70[PCL-PEG\textsubscript{1500}]-30[PCL], all proteins were released very fast, therefore fitting only 60% of drug release into model could not provide enough sampling points for meaningful conclusion.

Table 3. Coefficients of determination \( (R^2) \) and diffusional exponent \( (n) \) for the Korsmeyer-Peppas model. For highly swellable 70[PCL-PEG\textsubscript{1500}]-30[PCL], not enough sampling points were available (n.a)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>( R^2 )</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gos/Inulin_30[PCL-PEG\textsubscript{1500}]-70[PCL]</td>
<td>0.904</td>
<td>0.176</td>
</tr>
<tr>
<td>Gos/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.934</td>
<td>0.200</td>
</tr>
<tr>
<td>Ins/Inulin_30[PCL-PEG\textsubscript{1500}]-70[PCL]</td>
<td>0.976</td>
<td>0.430</td>
</tr>
<tr>
<td>Ins/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.957</td>
<td>0.385</td>
</tr>
<tr>
<td>Lys/Inulin_30[PCL-PEG\textsubscript{1500}]-70[PCL]</td>
<td>0.913</td>
<td>0.239</td>
</tr>
<tr>
<td>Lys/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.861</td>
<td>0.474</td>
</tr>
<tr>
<td>Lys/Inulin_70[PCL-PEG\textsubscript{1500}]-30[PCL]</td>
<td>n. a.</td>
<td>n. a.</td>
</tr>
<tr>
<td>CA/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.555</td>
<td>0.597</td>
</tr>
<tr>
<td>BSA/Inulin_70[PCL-PEG\textsubscript{1500}]-30[PCL]</td>
<td>n. a.</td>
<td>n. a.</td>
</tr>
</tbody>
</table>

4. Discussion

In this study, we synthesized novel phase-separated [PCL-PEG]-\( b \)-[PCL] multiblock co-polymers with varying PEG content and we showed that these polymers can be extruded at relatively low temperatures of 49 - 55 °C. Furthermore, we have shown that various proteins, spray-dried in the presence of inulin, can be incorporated in these polymers by HME without losing structural integrity. Moreover, it was found
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that the *in-vitro* release rate of the proteins from [PCL-PEG]-b-[PCL] implants increased with decreasing molecular weight of the protein and with increasing PEG content of the polymer.

DSC measurements showed that the multiblock co-polymers were phase separated with crystalline PCL domains, amorphous mixed PCL / PEG domains and crystalline PEG domains. However, in an aqueous environment, the crystallinity of PEG will be lost due to water uptake. The crystalline PCL domains, being insoluble in water, will act as physical cross-links and ensure controlled swelling. Water uptake experiments on polymer implants during degradation under *in-vitro* conditions showed that the swelling and degradation rate of the polymer was controlled by the [PCL-PEG\textsubscript{1500}] / [PCL] block ratio and thus by the PEG content. Swelling and degradation rate was limited for two polymers containing lower PEG amount (30[PCL-PEG\textsubscript{1500}]-70[PCL] and 50[PCL-PEG\textsubscript{1500}]-50[PCL]), while for the polymer with the highest PEG content (70[PCL-PEG\textsubscript{1500}]-30[PCL]), and thus with increased percentage of the amorphous block, the swelling and degradation rate of the polymer was much higher. Increased diffusion of water into the polymer resulted in a faster degradation of ester bonds, and thereby a faster decrease in mass and molecular weight of the polymer. It was observed that both 30[PCL-PEG\textsubscript{1500}]-70[PCL] and 50[PCL-PEG\textsubscript{1500}]-50[PCL] exhibited a low degradation rate. This is not surprising when keeping in mind that caprolactone based polymers slowly degrade *in-vitro* and *in-vivo* \([27,28]\). In contrast, 70[PCL-PEG\textsubscript{1500}]-30[PCL] lost 50 % of its mass already after 1 day. However, as explained before, it is unlikely that this is caused by degradation. Most likely, due to the small fraction of crystalline PCL block, the hydrophilic amorphous blocks exhibit unlimited swelling behavior and simply dissolve completely, leaving semi-crystalline PCL attached to remaining amorphous blocks. During degradation of this polymer, a small increase in average molecular weight of 70[PCL-PEG\textsubscript{1500}]-30[PCL] might be explained by dissolution of smaller molecular weight chains of this polymer.

The preservation of integrity of all proteins during both spray-drying and HME can be attributed to the stabilizing effect of inulin \([29–31]\) in combination with the relatively low extrusion temperature \([9]\).

Drug release from polymeric matrices depends on many factors. For bulk degrading polymers, the release is governed by drug solubility, drug diffusion, polymer swelling and / or polymer degradation, or a combination of these factors \([32]\). Furthermore, the rate of drug diffusion is dependent on the size of the drug compared to the polymer mesh size. Additionally, the particle size of the encapsulated drug is equally important for the release rate, as shown previously \([9]\). Therefore, in the present study, to avoid this additional influence of the particle size on the protein release, all protein / sugar samples were spray-dried to obtain powder particles with a
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uniform and narrow size distribution. The polymer mesh size is highly dependent on
the degree of swelling and the extent of degradation of the polymer matrix. Further,
the rate of drug diffusion depends on the diffusion constant of the drug in the water-
filled pores. For smaller molecules, diffusion is faster providing faster release.

To obtain more information on the mechanisms of the protein release from
the multiblock co-polymers, the release data were fitted into the Korsmeyer-Peppas
model. This model is suitable to analyze the release of drugs from pharmaceutical
products of which the release mechanism is not well known or of which the release is
governed by more than one mechanism [18]. In this model the value of n (diffusional
exponent) is considered to give an indication of the release mechanisms for different
group of matrices for moderately swelling polymers.

If the polymer mesh size is equal or larger than the size of the drug it seems
quite obvious that the drug can rapidly diffuse out of the polymer matrix, which
was observed for the smallest peptide Gos from 30[PEG-PEG 1500]-70[PCL]. For
this small molecule, the amount of PEG incorporated in the polymer matrix, and
therefore polymer swelling, did not play a role in release kinetics. The slightly higher
molecular weight protein Ins showed more continuous release from this polymer.
Lys, as a higher molecular weight protein, showed slower and continuous release
during the 160 days, which was the total time the release was monitored.

The Peppas model showed an n value of 0.45 for the release of Gos, Ins as
well as Lys from 30[PEG-PEG 1500]-70[PCL], suggesting a Fickian diffusion-based
release mechanism. This suggests that these proteins were sufficiently small to pass
through the polymer meshes [33]. The decreased release rate with increased drug size
can be explained by a combination of inherent slower diffusion in water for larger
proteins, but also by increased diffusion resistance through the polymer meshes,
as the polymer mesh size is a distribution. The diffusion controlled mechanism
is supported by the fact that 60 % of the protein was already released before any
molecular weight decrease occurred for 30[PEG-PEG 1500]-70[PCL] and that water
content was constant during the release experiment. This observation rules out the
occurrence of degradation or swelling controlled release mechanisms.

Similarly, the release of Gos and Ins from 50[PEG-PEG 1500]-50[PCL]
implants showed instantaneous release and equal kinetic behavior as 30[PEG-
PEG 1500]-70[PCL] based implants, which was confirmed by the similarity factor f_2.
This was also perceived for Lys, where the release from 50[PEG-PEG 1500]-50[PCL]
was slower than that found for the smaller proteins, but more rapid than the release
of Lys from the less swellable 30[PEG-PEG 1500]-70[PCL], demonstrating that
polymer swelling is important for the release of larger proteins. When increasing the
protein size to 29 kDa i.e. CA, a lag phase occurred, followed by continuous protein
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release. In addition, the molecular weight loss of this polymer was faster than that of 30[PCL-PEG\textsubscript{1500}]-70[PCL] indicating a faster degradation rate. Apparently, a certain degradation (or surface erosion) of the polymer matrix was needed before the protein could be released from the implants after which it was released by dissolution, which has also been previously shown by others [30].

Fitting the data into the Korsmeyer-Peppas model showed that Gos and Ins showed release from 50[PCL-PEG\textsubscript{1500}]-50[PCL] based on Fickian diffusion ($n < 0.45$), similar to 30[PCL-PEG\textsubscript{1500}]-70[PCL]. The Korsmeyer-Peppas model showed an $n$ value of 0.474 for release of Lys from 50[PCL-PEG\textsubscript{1500}]-50[PCL], suggesting anomalous transport, while for 30[PCL-PEG\textsubscript{1500}]-70[PCL] it was found that $n < 0.45$. In contrast to 30[PCL-PEG\textsubscript{1500}]-70[PCL], 50[PCL-PEG\textsubscript{1500}]-50[PCL] did show certain degradation during the period that 60 % of Lys was released. Water uptake and thus swelling remained constant during the release experiment. Therefore, besides diffusion, probably also degradation has contributed to the release of Lys from 50[PCL-PEG\textsubscript{1500}]-50[PCL]. The $n$ values for the release of CA from the same polymer ($n = 0.597$) suggested anomalous transport similar to Lys, where besides diffusion the release also depended on polymer degradation.

Obviously, when the polymer mesh size was smaller than the drug, the diffusion constant of the drug in the polymer matrix and thus the rate of release of the drug depended on the rate of degradation. Only when polymer chain scission continued causing the molecular weight of polymer to decrease, the protein started to diffuse out of the polymer matrix. Therefore, bulk degradation of the polymer and formation of interconnecting channels in the polymer matrix were necessary for larger molecular weight proteins to be released. However, BSA being the largest protein used in this study (66 kDa) showed no release at all from this polymer during 160 days. Most likely, degradation needs to proceed further causing more significant molecular weight and mass loss and therefore larger polymer mesh size for BSA to be released [33].

Our findings correlate well with studies done before [34], [35], where it was shown that the initial release of proteins from hydrogels depended on the hydrodynamic diameter of the encapsulated proteins, compared to the polymer mesh size, while subsequent release depended on polymer degradation. In a recent study by Lavin et al. the release of Ins, Lys, and BSA from wet spun microfibers was investigated. They confirmed that the release behavior was primarily driven by diffusion and depended on molecular weight, with smaller proteins releasing faster in the initial phase, and achieving a higher cumulative released fraction [16].

Evaluating the polymer with highest PEG content, 70[PCL-PEG\textsubscript{1500}]-30[PCL], both Lys and BSA were released within 8 days, whereas the larger molecular weight
protein BSA showed a slightly slower release rate than Lys. More than 50 % of mass loss indicated dissolution of the amorphous part of this polymer and consequently dissolution of the protein, which was assumed to be preferentially incorporated in the amorphous phase of the polymer matrix.

In conclusion, in this study we demonstrated that phase separated [PCL-PEG]-b-[PCL] multiblock co-polymers can successfully be used as a matrix for the incorporation of proteins by HME at temperatures as low as 49 - 55 °C, which could be beneficial for thermolabile proteins. As expected, all proteins retained their structural integrity, which could be ascribed to incorporation into the glassy matrix of inulin [30]. Hence, we were able to demonstrate that indeed molecular weight of the protein and PEG content of the polymer are factors of utmost importance in regulating protein release from polymeric matrix implants. Careful matching of both aspects is essential in designing the optimal delivery depot with tailored protein release.

Acknowledgments

This research has been performed within the framework of the Northern Drug Targeting and Delivery Cluster (EFRO Grant).

Supporting information:

*Figure S1. SDS-PAGE results of BSA samples. Lanes: 1 Molecular weight marker; 2 and 3 extracted BSA from 50[PCL-PEG1500]-50[PCL]; 4 and 5 extracted BSA from 70[PCL-PEG1500]-30[PCL]; 6 native BSA.*
### Table S1. Actual protein loading of implants (%) as determined by extraction (n = 6).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Average loading (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1   Gos/Inulin_30[PCL-PEG\textsubscript{1500}]-70[PCL]</td>
<td>1.10 ± 0.09</td>
</tr>
<tr>
<td>2   Gos/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>1.10 ± 0.08</td>
</tr>
<tr>
<td>3   Ins/Inulin_30[PCL-PEG\textsubscript{1500}]-70[PCL]</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>4   Ins/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.77 ± 0.02</td>
</tr>
<tr>
<td>5   Lys/Inulin_30[PCL-PEG1500]-70[PCL]</td>
<td>0.98 ± 0.03</td>
</tr>
<tr>
<td>6   Lys/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>1.03 ± 0.03</td>
</tr>
<tr>
<td>7   Lys/Inulin_70[PCL-PEG\textsubscript{1500}]-30[PCL]</td>
<td>1.06 ± 0.01</td>
</tr>
<tr>
<td>8   CA/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.85 ± 0.02</td>
</tr>
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<td>9   BSA/Inulin_50[PCL-PEG\textsubscript{1500}]-50[PCL]</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>10  BSA/Inulin_70[PCL-PEG\textsubscript{1500}]-30[PCL]</td>
<td>0.89 ± 0.05</td>
</tr>
</tbody>
</table>
References:

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