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Research paper

In situ forming parenteral depot systems based on poly(ethylene carbonate): Effect of polymer molecular weight on model protein release

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ABSTRACT

The objective of this study was to investigate the effect of molecular weight (MW) on the drug release from poly(ethylene carbonate) (PEC) based surface-eroding in situ forming depots (ISFD). In phosphate buffered saline (PBS) pH 7.4, 63.7% of bovine serum albumin BSA was released from high MW PEC of 200 kDa (PEC200) in DMSO (15%, w/w) in 2 days, while during the same time period, the release of BSA from PEC41 samples was only 22.5%. At higher concentrations of PEC41 (25%, w/w), the initial burst was further reduced, and even after 6 days, only 16.3% was released. Compared to depots based on PEC200, there was lower rate of solvent release, slower phase inversion, and a denser surface in PEC41 samples. An expansion in size of PEC41 depots suggested that the polymer barrier of PEC41 impeded the diffusion of solvent out of the samples effectively. In conclusion, the initial burst of protein from ISFD of PEC41 was significantly reduced, which would be a promising candidate as polymeric carrier.

1. Introduction

Carbonic acid derivatives of poly(ethylene carbonate) (PEC) and poly(trimethylene carbonate) (PTMC) are known not to degrade *in vitro* by hydrolysis in the pH range of 1–12, while their *in vivo* degradation shows all the characteristics of surface erosion, where the molecular weight (MW) of the polymer remains unchanged during the entire period of biodegradation [1–6]. This is thought to occur preferentially from the polymer surface in close contact with cells of the immune system, including macrophages and polymorphonuclear leukocytes (PMN) [3,6]. Also the *in vivo* degradation of PEC is much faster than PTMC [2]. Subcutaneous implants of PEC disappeared within weeks to months from the body, which is thought to be optimal for parenteral sustained drug delivery systems (DDS) [7]. Based on these beneficial properties, PEC has been widely applied in different DDS such as nanoparticles [8], microparticles [1,2], and in situ forming depots (ISFDs) [9].

Biodegradable ISFDs represent an attractive alternative to implants and microspheres [10,11]. The polymer solution undergoes a process of phase inversion after administration into the body at the site of interest, whereby polymer solution is converted into a solid/semisolid depot by solvent diffusion or temperature change [10,11]. Consequently, an ISFD using PEC with MW of 247.8 kDa based on in situ polymer precipitation for drug delivery was developed, although both the initial burst and the viscosity of polymer solution were found to be suboptimal [9].

In this study, to obtain the PEC based ISFD system with low viscosity and decreased initial burst of drug for further therapeutic applications, PEC polymers with a higher MW of 200 kDa (PEC200) and a lower MW of 41 kDa (PEC41) were used to prepare the ISFDs with bovine serum albumin (BSA) as a model protein. The mechanism of various initial release rates of BSA from the PEC depots was also investigated.

2. Experiments

2.1. Materials

Poly(ethylene carbonate) (PEC) with a MW of 200 kDa (PEC200) was synthesized as previously described [7]. PEC with a MW of 41 kDa (PEC41) was produced in hot water [12]. Fluorescein isothiocyanate conjugate bovine serum albumin (FITC-BSA) was purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals and solvents were of analytical grade and used as received.

2.2. Viscosity of polymer solutions

15–30% (w/w) of PEC41 in DMSO ($n = 3$) mixtures were investigated to determine their dynamic viscosity using a HAAKE RheoStress 1 plate-cone rotational viscosimeter (Thermo Fisher

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Scientific Inc., Karlsruhe, Germany) with a sensor of 60 mm diameter, a slit of 0.052 mm, and an angle of 1°. Measurements were performed at 20 °C with the linear shear rate ranging from 0.1 to 25 s^{-1} in 2 min. The dynamic viscosity of the polymer solution was represented by the slope of the rheogram.

2.3. Initial release

5 mg of FITC-BSA was dispersed in 1 g of 15% (w/w) of PEC200 and 15% and 25% of PEC41 DMSO solutions, respectively, by an Ultra-Turrax T25 (IKA, Staufen, Germany) at 24,000 rpm for 2 min. Then, 100 mg of the suspension was added into 1 mL of PBS pH 7.4 containing 0.02% NaN_3 in a 2 mL glass vial at 37 °C in an KS 4000 shaker (IKA, Staufen, Germany) at 50 rpm ($n = 3$). 0.6 mL of the medium was replaced with blank PBS solution at 3 h, 24 h, and 48 h and afterward at predetermined time points with an interval of 2 days. The supernatant was diluted by PBS buffer further, and 200 μL of the samples was added into a 96-well plate. The amount of FITC-BSA released was determined by an OPTIMA FLUOstar fluorescence microplate reader (BMG Labtech, Offenburg, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The calibration curve was established in the range of 0.2–2 μg/g ($R^2 > 0.99$). Experiments were conducted in triplicates.

2.4. Scanning electron microscopy (SEM)

SEM was performed to investigate the internal morphology of PEC ISFD samples after they were injected into aqueous phase. Briefly, 100 mg of various PEC DMSO solutions was injected into 1 mL of PBS buffer at 37 °C. 0.6 mL of the medium was refreshed as described above. At day 1.5 and day 14, the samples were washed carefully with distilled water and freeze-dried for 3 days at 3 mbar and −40 °C. Afterwards, the ISFD samples were sputter-coated with gold and imaged on a JSM-7500F SEM (JEOL, Tokyo, Japan).

2.5. In vitro release of solvent

15% of PEC200 and 15% and 25% of PEC41 were dissolved in DMSO (w/w), and then, 100 mg of the organic PEC solution was added into 1 mL of PBS buffer solution at 37 °C ($n = 3$). At predetermined time points, 0.6 mL of medium was replaced with blank PBS buffer solutions. The amount of DMSO was determined by HPLC according to a previous report [9].

2.6. Critical water concentration

The critical water concentration necessary to initiate phase inversion of the polymer solution was investigated by adding PBS pH 7.4 solutions to PEC200 and PEC41 dissolved in DMSO (w/w) ($n = 3$) at room temperature. The polymer concentration was 1% (w/w) in the mixture of water and DMSO. After the buffer was added, the solutions were magnetically agitated at 600 rpm for 1 h. Afterwards, the samples were centrifuged at 10,000*g* for 10 min, and then, the supernatant was removed. The precipitate was washed with distilled water thoroughly and lyophilized for 3 days at 3 mbar and −40 °C. The dry precipitate was weighed, and the mass was compared with the total injected mass to obtain % of PEC precipitation as a function of % water in DMSO.

2.7. Water swelling of ISFD

100 mg of various PEC DMSO solutions containing 0.5% of FITC-BSA was injected into 1 mL of PBS at 37 °C. The medium was replaced as described above in the initial release section. At preset time points, the surfaces of ISFD samples were blotted and the masses of the specimens were recorded ($n = 3$ per time point).

3. Results and discussion

3.1. Viscosity of polymer solutions

It has been suggested that liquids with dynamic viscosities of <600 mPa s could be suitable as injectable formulations using needle sizes of 23 G or less [13]. A previous report indicated that 11.2% (w/w) of PEC with a MW of 247.8 kDa in DMSO had a dynamic viscosity of >2, 000 mPa s; however, high initial bursts of BSA of more than 70% in 24 h were observed [9]. We hypothesized that low MW PEC41 in DMSO solution would show a lower dynamic viscosity making injectable formulations at higher polymer concentrations possible. As compiled in Table 1, this objective was achieved, and even 30% (w/w) solutions of PEC41 did not exceed the 600 mPa s threshold. Hence, an ISFD based on PEC41 with high polymer concentrations could be used for parenteral drug delivery.

3.2. Initial burst of model protein FITC-BSA

In this investigation, high MW PEC200 and low MW PEC41 in DMSO were loaded with FITC-BSA, and the initial drug release (burst) was compared in a non-degrading PBS pH 7.4 medium at 37 °C. As demonstrated in Fig. 1, PEC200 at the highest injectable concentration of 15% (w/w) led to a drug burst of >40% in 3 h, and furthermore, 63.7% of BSA was released after 2 days by diffusion. This release profile is not attractive for parenteral protein delivery. By contrast, for an ISFD of PEC41 at the same polymer concentration, only 12.2% and 22.5% of the protein was released after 3 h and 2 days, respectively (Fig. 1). With an increase in PEC41 concentration to 25%, the initial release was reduced further and only 16.3% of FITC-BSA was released after 6 days (Fig. 1). Collectively these data demonstrate that PEC41 would have beneficial effects not only on injection viscosity but also on the initial drug burst of a model protein.

3.3. Morphological analysis using scanning electron microscopy (SEM)

A reduced initial burst has been thought to be caused by a denser structure of the ISFD [14–16]. Thus, the morphology of depots was studied by scanning electronic microscopy (SEM). Fig. 2A shows macro-voids in the internal structure of the depots from 15% of PEC200 with a thin and porous polymer surface after 1.5 days in PBS, pH 7.4. In contrast, 15% of PEC41 based depots revealed a dense shell-like structure, which was less porous than that of the PEC200 samples (Fig. 2B). The ruffled samples for 25% of PEC implicated that there was still residual DMSO (Fig. 2C), which had the lowest initial release compared with the samples from 15% of PEC200 and PEC41. After incubation in water for 14 days, the structure of the 25% PEC41 ISFD sample was solid and not ruffled (Fig. 2D). Numerous micro-voids and some macro-voids can be seen in the depots, which also showed a dense shell-like structure, however, much thicker than that of 15% of PEC41. The dense

Table 1
Dynamic viscosities of PEC41 DMSO solutions at 20 °C ($n = 3$).

Polymers	Concentrations of PEC in DMSO (%, w/w)	Dynamic viscosities (mPa s)
PEC41	15	67 ± 12
PEC41	20	90 ± 23
PEC41	25	360 ± 35
PEC41	30	520 ± 41