Protein Release from Poly(ortho ester) Extruded Rods

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SUMMARY: Poly(ortho ester) rods containing 15 wt% FITC-BSA were prepared by extruding an intimate mixture of finely powdered polymer and protein at a temperature where protein activity is retained. After an induction period, linear in vitro release kinetics were obtained with concomitant polymer weight loss.

Introduction

Proteins contain complex tertiary structures that are essential for their biological activity, and this tertiary structure must be maintained during incorporation into the delivery device while in the device and upon release from the device. It is generally assumed that proteins are stable in organic solvents such as dichloromethane, but there can be some unfolding[1)] and when a significant amount of water is added to the organic solvent, proteins will denature and then aggregate[2)].

However, because in the absence of water proteins are resistant to heat denaturation[3)], thermal fabrication methods are a viable method for fabricating delivery devices. In this manuscript we describe the fabrication of protein delivery devices by extrusion and present polymer erosion kinetics and *in vitro* release kinetics of a model protein, bovine serum albumin (BSA).

Polymer Synthesis

Successful development of protein delivery devices prepared by solventless extrusion require a polymer that can be extruded at temperatures low enough so that protein activity is not compromised, and where polymer erosion and protein delivery rates are closely coupled and can be controlled to give desired release profiles.

We have described such a polymer[4)], have carried out extensive studies to elucidate the mechanism of hydrolysis[5)] and have used this polymer to develop a variety of drug delivery

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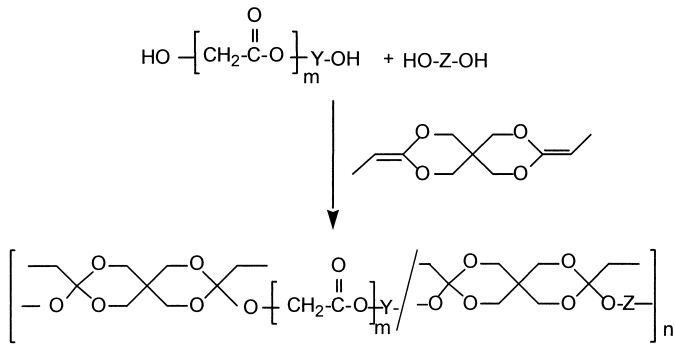
devices[6]. The polymer is readily prepared by the addition of various diols to a bisketene acetal, 3,9-diethylidene 2,4,8,10-tetraoxaspiro[5.5]undecane as shown in Scheme 1.



Scheme 1

The synthesis is well understood and polymers ranging in molecular weight from a few thousands to well over 100 000 can be routinely and reproducibly prepared[6]. We have also shown that the polymer can be stored at room temperature provided that moisture is rigorously excluded.

Experimental

Device Fabrication - Polymers were reduced to a fine powder by either cryomilling, or by spray drying and then intimately mixed with micronized protein by using a mortar and pestle. Rods were obtained by extruding mixtures of polymer and 15 % of fluorescein isothiocyanate – BSA (FITC-BSA), using a laboratory ram extruder described previously[7]. The mixture of drug and polymer was introduced into a 10 mm I.D. barrel, in which a piston (d = 10 mm) was inserted and then moved into the heated barrel using air pressure. The 1-mm diameter extruded cylinders were cut into 10 mm lengths.

***In Vitro* Drug Release** - Rods were placed in 25-ml capped glass bottles (one rod per bottle), containing 20 ml of 0.01 M phosphate saline buffer (pH 7.4). The temperature was maintained at 37 °C and the bottles were maintained under moderate agitation in a horizontal shaker (type 3022, Gesellschaft für Labortechnik, Burgwedel, Germany). After desired time intervals, 3 ml of the buffer were collected for protein content analysis and replaced with 3 ml fresh buffer. Protein content was quantified spectrophotometrically at 494 nm.

Weight Loss and Molecular Weight Determination - At defined time intervals, rods were removed from the buffer and dried. Weight-average molecular weight measurements were performed using a Waters 150C high-pressure SEC equipped with four Styragel columns mounted in series. The columns used were a Waters HR 1 column (effective molecular weight range 500-5000), a Waters HR 2 column (500-20 000), a Waters HR 3 column (500-30 000) and a Waters HR 4 column (5 000-500 000). Tetrahydrofuran (analytical grade) was used as the mobile phase; the injected sample volume was 200 μl, the sample concentration was 0.1 wt %, the temperature of the columns was 30 °C and the flow rate was 1.0 ml/min. Injections were performed using a Waters 717 autosampler. To calibrate the system, monodisperse polystyrene standards of the following molecular weights were used: $5.0x10^2$, $2.63x10^3$, $5.97x10^3$, $9.1x10^3$, $1.81x10^4$, $3.79x10^4$ and $3.55x10^5$ (Tosoh Corporation, Japan).

Results

As already described[4-6], the nature of Y and Z controls polymer properties and two important polymer thermal properties as a function of Y and Z are shown in Table 1.

Figure 1 shows release of FITC-BSA from a polymer prepared from a 100/95/5 mixture of 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane, pentane-1,4-diol and (6-hydroxyhexyl)-glycolide, extruded at 70 °C. Three major features are notable. First, there is only a minimal burst despite the fact that 15 wt% of a water-soluble material has been incorporated, second, there is a significant induction period, and third, polymer weight loss and BSA release occur concomitantly.

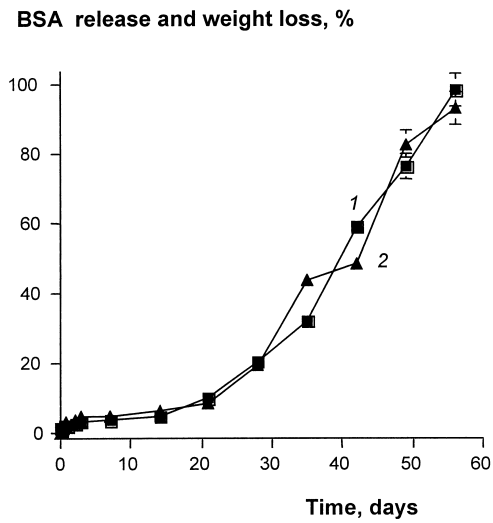
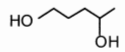
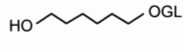
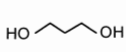
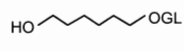
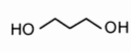
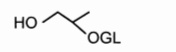
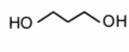
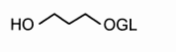
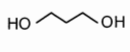
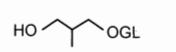
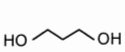
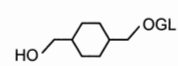
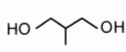
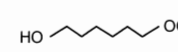
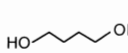
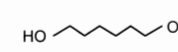
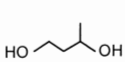
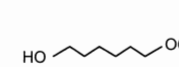


Fig. 1: Weight loss (*1*) and release of FITC-BSA (*2*) from a poly(ortho ester) prepared from 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane, pentane-1,4-diol and (6-hydroxyhexyl)-glycolide, 100/95/5. (Rods, 1x10 mm, extruded at 70 °C; 0.01 M phosphate-buffered saline, pH 7.4, 37 °C.)

Table 1. Polymer composition, glass transition temperature (T_g) and minimum extrusion temperature (MET) as a function of polymer composition.

Diol[a] (95 mol %)	(Hydroxyalkyl)glycolide[b] (5 mol %)	T_g (°C)	MET (°C)
		46.5	68.0
		46.5	50.0
		60.5	90.0
		58.2	85.0
		57.3	80.0
		60.0	90.0
		48.6	65.0
		47.8	70.0
		48.7	60.0

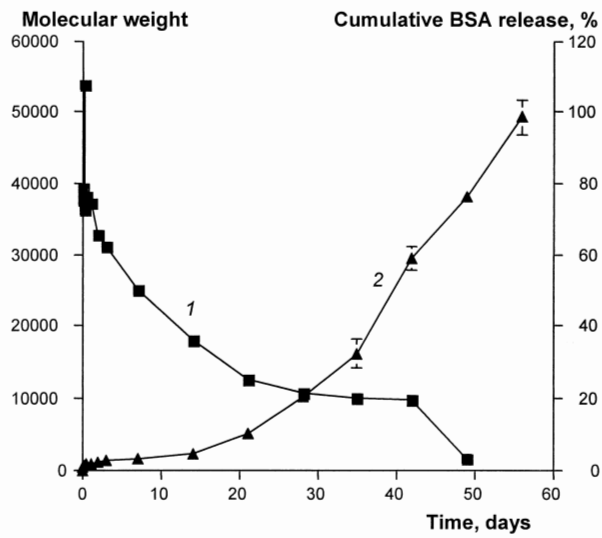


Fig. 2: Change in molecular weight (*1*) and release of FITC-BSA (*2*) from poly(ortho ester prepared from 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5]undecane, pentane-1,4-diol and (6-hydroxyhexyl)glycolide (100/95/5). (For details, see Fig. 1.)

Figure 2 shows BSA release and changes in molecular weight of the remaining polymer. The data indicate that there is a continuous reduction in polymer molecular weight during the induction period and that no BSA is released until the molecular weight has decreased to about 20 000 from the original 61 500. After that, there is only a modest decline in molecular weight with concomitant zero-order release of BSA.

The possibility that the induction period is a function of polymer molecular weight has been considered and investigated by comparing induction periods of two polymers, a 61 500 molecular weight material and a 12 000 material. Since, as shown in Fig. 3, the induction period remains unchanged, the induction period is not molecular-weight-dependent. Further, at least for the two molecular weights investigated, neither polymer erosion nor BSA release is significantly affected.

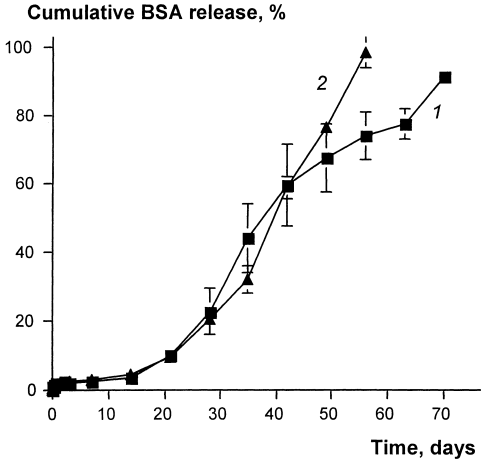


Fig. 3. Effect of polymer molecular weight on induction period for a poly(ortho ester) prepared from 3,9-diethylidene-2,4,8,10-tetraoxaspiro[5.5] undecane, pentane-1,4-diol and (6-hydroxyhexyl)glycolide, 100/95/5. Polymer molecular weight: (*1*) 12 000, (2) 61 500. (For details, see Fig. 1.)

Discussion

When proteins are incorporated into a poly(ortho ester) by extruding an intimate mixture of micronized protein and finely ground polymer into thin rods which are then placed in a pH 7.4 buffer at 37 °C, FITC-BSA, is released at linear kinetics after a significant induction period. Further, because protein release and polymer erosion occur concomitantly over the whole release period, release of the protein occurs by a process that is completely erosion-controlled. Three important implications of this process are (a) protein release will occur without a significant initial burst and by linear kinetics, (b) control of the erosion process will control protein release kinetics, and (c) when all the protein has been delivered, no polymer remains. The release profile shown in Fig. 1 is significantly different from that observed for protein

release from poly(lactic acid-*co*-glycolic acid) copolymers which initially have a porous structure that becomes even more so as the erosion process proceeds, and where protein release is largely governed by diffusion with a significant initial burst. For example, release of recombinant human growth hormone from a 50/50 poly(lactic acid-*co*-glycolic acid) microspheres is characterized by a 35% initial burst, followed by reasonably linear kinetics for 14 days until 80% had been released, with no further release noted up to one month[8].

The extrusion method has been validated by using the zinc salt of recombinant human growth hormone, incorporated into the polymer at 15 wt%, and extruding a rod at 70 °C. Protein integrity following extraction from the rod was assayed by high-performance liquid chromatography. Preliminary data show that there was only a slight loss of activity despite the fairly high extrusion temperature that was used. Additional extrusion and protein activity assays are currently ongoing.

References

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