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Pulsatile protein release from a laminated device comprising of polyanhydrides and pH-sensitive complexes

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Abstract

A laminated device comprising of polyanhydrides as isolating layers and pH-sensitive complexes as protein-loaded layers was designed to deliver proteins in a pulsatile manner. Poly(sebacic anhydride)-*b*-polyethylene glycol (PSA-*b*-PEG) and poly(trimellitylimidoglycine-*co*-sebacic anhydride)-*b*-polyethylene glycol (P(TMA-gly-*co*-SA)-*b*-PEG) were synthesized as isolating layers for their good processing properties at room temperature and suitable erosion duration. During the erosion period, pH of the dissolution fluid decreases to a low value (3.8–5.8). Poly(methacrylic acid)/polyethoxazoline (PMAA/PEOx) complex was used as protein-loaded layers, which could dissociate and release model proteins, Myoglobin (Mb) and Bovine Serum Albumin (BSA), at pH 7.4 while become stable and retained the drugs below pH 5.0. The protein release from the device showed a typical pulsatile fashion. The lag time prior to the pulsatile protein release correlated with the hydrolytic duration of the polyanhydrides, which varied from 30 to 165 h by selecting polyanhydride type and isolating layer thickness. In addition, the pulse duration could be adjusted from 18.5 to 40 h by varying the mass of the complex. The results can be attributed to the synergistic effects between the degrading polyanhydrides, pH-sensitive complexes and proteins. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Polyanhydrides; pH-sensitive complexes; Pulsatile protein release; Synergistic effect

1. Introduction

In the last few years, major advances have been made in therapeutics with the development of genetic engineering allowing the production of many pharmacologically active peptides and proteins. The high pharmacological potency of these drugs is counterbalanced by their poor penetration through the physiological barriers, their fragility together with their therapeutic uses (Banga and Chien, 1988). In different strategies proposed to overcome these problems, entrapment of peptides or proteins into biodegradable polymers or hydrogels which would allow release of these drugs over an extended period of time is promising (Hayashi, 1994). There is growing evidence that continuous delivery may not be the optimal delivery manner for all these drugs, but that pulsed delivery may be preferred in many cases (Mathews et al., 1983; Clark et al., 1985; Brewitt and Clark, 1988). For example, it has

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been demonstrated clinically that pulsatile administration of Gonadotropin releasing hormone (GnRH) and Luteinizing hormone-releasing hormone (LHRH) is essential in the restoration of normal reproductive function (Santoro et al., 1986; Giusti and Cavognaro, 1991). Two kinds of pulsed release systems have been proposed. One is that releases drugs in response to some environmental or physiologic variables (Kost, 1990; Langer, 1990). The other is preprogrammed delivery system that delivers drugs depending on the device structure. Wuthrich et al. (1992) synthesized an erodible, ointment-like poly(ortho ester) as carriers for proteins and found that proteins were released in a delay, pulsed fashion. Bae et al. (1991) described interpenetrating polymer networks that delivered drugs in a biphasic manner. Jimoh et al. (1995) proposed an implantable capsule capable of bursting at a predetermined time for pulsed delivery of hormones using gas generating compositions as mechanistic tools. Krõgel and Bodmeier (1998) developed a device consisting of an impermeable capsule body and an erodible plug wherein the pulsatile release could be controlled by the erosion properties of a compressed or congealed plug placed within the opening of the capsule. All these schemes would probably be most suitable for therapies in which only one pulse was needed. Fujioka et al. (1987) designed a laminated device composed of interferon-free collagen layers and interferon-loaded collagen layers, which could achieve several pulses from one formulation. However, the pulsatile protein release pattern cannot be finely modulated by using water-soluble polymers, due to their quick dissolution nature. The objective of this paper is to develop and evaluate an alternative laminated device for pulsatile protein delivery, which essentially comprises of biodegradable polyanhydrides and pH-sensitive complexes. Here, polyanhydrides are used as isolating layers so that lag time of protein release can be modulated within a broad period range. The pH-sensitive complexes are adopted as protein-loaded layers in order to maintain protein bioactivity since the direct contact of proteins with acidic microclimate created during polyanhydride degradation and hydrophobic surface of polyanhydrides may be harmful for some protein bioactivity (Powell, 1996; Mäder et al., 1997b). The complexation of proteins with polyelectrolytes may enhance their stability under various harsh conditions, such as, organic solvents, low pH environment and high temperature (Kokufuta and Takahashi, 1990; Kudryashova et al., 1997; Dumitriu and Chornet, 1998). More importantly, by the unique synergistic effect between the degrading polyanhydrides, pH-sensitive complexes and proteins, the proteins release in a pulsatile manner and the release pattern, i.e. both lag time of protein release and each pulse duration, could be easily tailored.

2. Materials and methods

2.1. Materials

PSA-*b*-PEG and P(TMA-gly-*co*-SA)-*b*-PEG were synthesized by melt-condensation copolymerization (Jiang and Zhu, 1999). PMAA (M_w = 380 000) was synthesized through free-radical polymerization of methacrylic acid in this lab. Polyethoxzoline (PEOx, $M_w = 5\,000\,000$) was purchased from PolyScience Co. Myoglobin (Mb, from horse heart), fluorescein isothiocyanate Bovine Serum Albumin (FITC-BSA) and FITC-dextran ($M_w = 67\,200$) were supplied by Sigma Chemical Co. Poly(lactide-*co*-trimethyl carbonate) (PLTMC, 30:70 by molar) was synthesized by ring-opening copolymerization of lactide and trimethyl carbonate using $Sn(Oct)_2$ as catalyst in this lab (Cai and Zhu, 1997).

2.2. Erosion of polyanhydrides

Polyanhydride discs (2.8 mm in diameter and 2.2 mm in thickness) were prepared by compression molding of the polyanhydride powder (< 10 μm) with a homemade apparatus at 100 kg/cm^2 and room temperature for 5 min, then coated in PLTMC film (about 0.15 mm in thickness) with one open-end left. The degradation was performed in a daily-changed 0.1 M phosphate buffer solution at pH 7.4 and 37°C. The erosion rate was measured by the change of dry weight of the polymer samples. pH of the dissolution fluid

was determined with a pH meter at definite time intervals.

2.3. Preparation of PMAA/PEOx complex

Complexes were prepared according to the method described by Kwon et al. (1991). Briefly, 50 mg of Mb or FITC-BSA was dissolved in 100 ml of 2.5 mg/ml of the mixed PMAA/PEOx solution (PMAA/PEOx ratio was 0.869 by weight, corresponding to a 1:1 ratio of repeating units, pH 6.0) and the complex was formed by decreasing the pH of the solution to 4.0 with 0.1 M HCl solution under magnetic stirring. The precipitates were then isolated, washed with double-distilled water and dried in vacuum at room temperature. Complex yield: $>90\%$. Protein entrapment efficiency: $>95\%$. Protein loading percent was 9.4% for Mb, 9.52% for FITC-BSA. The complex was ground with a mortar and pestle and sized by use of sieves. Granules with diameter smaller than 10 μm were collected and used for following experiments.

2.4. Mb or FITC-BSA release from PMAA/PEOx complex

A total 20 mg of the protein-loaded complex was compressed into discs (2.8 mm in diameter and ca 2.0 mm in thickness) at 100 kg/cm^2 and room temperature for 10 min, then coated in PLTMC film with one open-end left. The capsulated complex was soaked in 5 ml of 0.1 M buffer solution with various pH at 37°C. The buffer solution was removed and fresh solution was added back periodically. Mb release was monitored at 420 nm using an UV–Vis spectrophotometer (Shimatz-1201) and FITC-BSA was measured with a fluorescence spectrophotometer (Hitachi F-4000).

2.5. Preparation of the laminated device

Each layer, including polyanhydrides and complexes, was individually prepared (2.8 mm in diameter) as above described, then compressed together into an alternate cylindrical preparation by compression molding at 100 kg/cm^2 and room temperature for 10 min and finally coated in PLTMC film with one open-end left. Fig. 1 shows the structure of the model device.

2.6. Protein release from the laminated device

The laminated device was immersed in 0.1 M pH 7.4 phosphate buffer and agitated at 37°C and 100 rpm in an environmental incubator shaker. The dissolution fluid was removed and fresh solution was added back periodically. Mb release was measured by the absorbance of the buffer solution at 420 nm and FITC-BSA delivery was detected by its fluorescence intensity in the buffer solution. pH of the dissolution fluid was monitored with a pH meter.

3. Results and discussion

3.1. Synthesis of polyanhydride

It is difficult for poly(lactide-*co*-glycolide) (PLGA) to be fabricated into dense cylindrical device at room temperature arising from its relatively high T_g (>50°C). In the present experiments, PSA-*b*-PEG (PSP) (80:20, 65:35, molar ratio) and P(TMA-gly-*co*-SA)-*b*-PEG (PTSP) (50:30:20) were selected as isolating layers for their good processing properties as well as suitable erosion duration (Jiang and Zhu, 1999). Table 1 lists the essential parameters of the polyanhydrides. It can be seen that all the polymers have low T_g (<12°C) and crystallinity ($<23\%$), which enables the polymers be easily

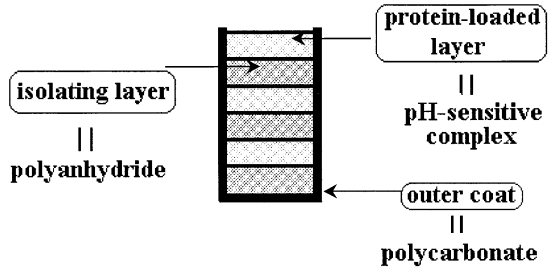

Fig. 1. Structure of the model laminated device for pulsatile protein release.

Table 1
Characteristics of polyanhydrides

Polymer	M_n	M_w	T_g (°C)	T_m (°C)	Crystallinity[a] (%)
PSA-*b*-PEG200[b] (80:20[c])	7800[d]	9600	−40	72.2	23.1
PSA-*b*-PEG 200 (65:35)	3100	5900	−46	66.3	3.0
P(TMA-gly-*co*-SA)-*b*-PEG 200 (50:30:20)	3200	5600	12	ND[e]	0

[a] Crystallinity is calculated according to literature (Mathiowitz et al., 1990).
[b] The number behind PEG refers to the molecular weight of PEG segment.
[c] Molar ratio.
[d] Measured by GPC, THF as solvent.
[e] ND, not detectable.

compressed into dense cylindrical implant at room temperature.

Erosion behavior of the polyanhydrides is shown in Fig. 2. It can be seen that hydrolytic duration relates to polymer nature. PSP (80:20) degrades completely within 140 h, 100 h for PSP (65:35) and 30 h for PTSP (50:30:20). Increasing in PEG content in PSP results in an increase in polymer hydrophility, which accelerates the polymer erosion. However, when PEG content in the polymer is above 60%, the polyanhydrides are hydroscopic and it is difficult for them to be fabricated into cylindrical implant. Introduction of TMA-gly segments into PSP can further increase erosion rate of the polymer while maintaining its mechanical strength due to hydrophility and rigid structure of TMA-gly (Staubli et al., 1990).

Fig. 3 demonstrates pH variation of dissolution fluid during the polyanhydride erosion. All of them show that the pH is decreased by release of small fragments during erosion. For example, pH varies from 4.0 to 5.8 for PSP (80:20), 4.2 to 5.6 for PSP (65:35) and 3.05 to 5.8 for PTSP (50:30:20). They also have a distinct minimum that marks the time with the highest release rate of fragments, which is similar to the results reported by Göperferich and Langer (1993).

3.2. Protein release from PMAA/PEOx complex

PMAA/PEOx was selected as protein-loaded layers in the present work due to their excellent pH-sensitivity and high hydrophobicity. Fig. 4 shows Mb release profiles from PMAA/PEOx complex coated in PLTMC film with one open-end left. It can be seen that pH has a great effect on the protein release. At pH 7.4, Mb releases linearly within ca 48h, whereas below pH 5.0, Mb release rate is rather low (less than 4% within 48 h). The erosion behavior of PMAA/PEOx is nearly identical to that of Mb release, i.e. the complex remains stable below pH 5.0, while becoming dissociable at pH 7.4 (results not shown). In our previous study, we found that Mb could interact with PMAA by electrostatic forces at low pH and form a water-soluble complex that further interacted with PEOx and precipitated from solution at low pH (<5.0), yielding Mb/PMAA/PEOx ternary complex (IEP of Mb is 6.99) (unpublished results). Since the complex remains

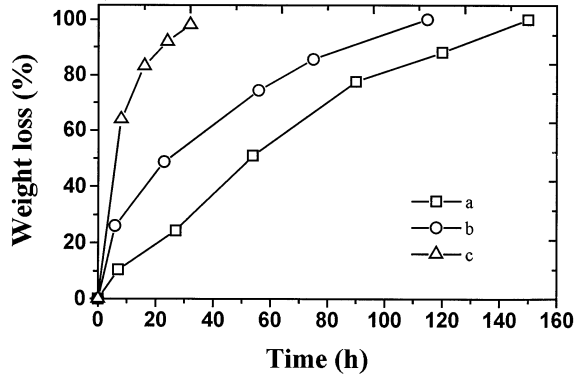

Fig. 2. Weight loss of polyanhydrides during erosion in 0.1 M phosphate buffer at 37°C. All the cylindrical samples were coated in PLTMC film with one-end left. (a) 20 mg of PSA-*b*-PEG200 (PSP) (80:20, molar ratio); (b) 20 mg of PSP (65:35); (c) 20 mg of P(TMA-gly-*co*-SA)-*b*-PEG200 (50:30:20, molar ratio) (PTSP) ($n=3$).

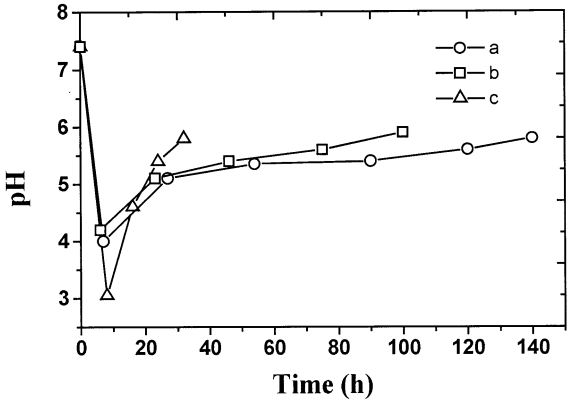

Fig. 3. pH variation of buffer solution during polyanhydride erosion. All the cylindrical samples were coated in PLTMC film with one-end left. (a) 20 mg of PSP (80:20); (b) 20 mg of PSP (65:35); (c) 20 mg of P(TMA-gly-*co*-SA)-*b*-PEG200 (50:30:20) (PTSP) ($n = 3$).

stable and there are electrostatic interactions between Mb and PMAA below pH 5.0, the protein is retained in the pH range. The dissociation of PMAA/PEOx complex results in the protein release at pH 7.4. The linearity of complex dissociation and Mb release with time implies that this process only occurs through erosion of the opening end since zero-order kinetics are characteristic of surface erosion in slab-type polymer matrices (Hopfenberg, 1976). Similar results were reported by Kwon et al. (1991). It was observed that

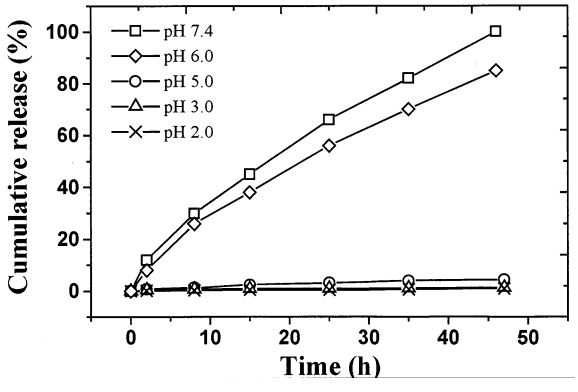

Fig. 4. Mb release from PMAA/PEOx complexes. A total of 20 mg of complex disc was coated with PLTMC film with one open-end left ($n = 3$).

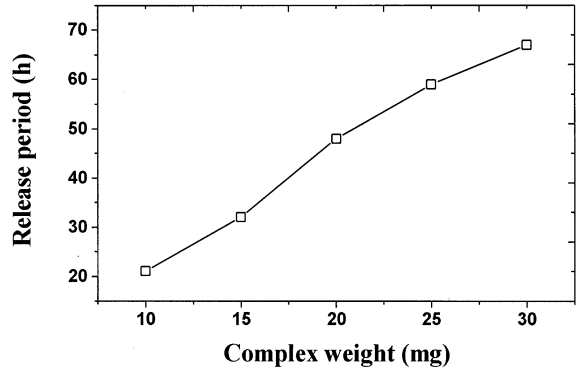

Fig. 5. The influence of PMAA/PEOx complex mass on the Mb release period from the complex disc coated in PLTMC film with one open-end left ($n = 3$).

equilibrium swelling of the capsulated complex is reached within ten days and the equilibrium water content is ca 8% by weight, at pH 4.0, which indicates the high hydrophobicity of PMAA/PEOx complex. The high hydrophobicity results in its surface-erosion characteristics at pH 7.4. Fig. 5 shows the influence of PMAA/PEOx complex mass on Mb release period from the coated complex disc at pH 7.4, which indicates that the Mb release duration can be finely adjusted from ca 20 to 70 h simply by varying the complex mass. The release of FITC-BSA from PMAA/PEOx complex is nearly identical to that of Mb (IEP_{BSA} is 4.9).

In contrast to the pH-sensitive release of the proteins from PMAA/PEOx complex, FITC-dextran release is independent of buffer pH and the complete release period is ca 6 h, resulting from the lack of electrostatic interactions with PMAA.

3.3. Pulsatile protein release from the laminated device

The polyanhydrides and the protein-loaded pH-sensitive complexes were compressed into a laminated cylindrical preparation, then coated in PLTMC film with one open-end left. PLTMC (30:70) is selected as outer coat due to its slow erosion (weight loss is ca 10% within 3 months), high hydrophobicity (equilibrium water content is ca 3%) and low permeation to proteins.

It was observed in vitro test that the laminated device remained its original shape, but the inner alternate layers gradually lost from the opening end of the device. The results of protein release are shown in Fig. 6, which is expressed as the cumulative protein release of each pulse with time. It can be seen that in all the cases the proteins release in a pulsatile manner. For example Mb releases from the device I (the device composition is shown in Table 2) with three separated pulses (each pulse ca 40 h) after that followed by a lag time of ca 98 h with no Mb release (Fig. 6(B)).

It is noticed that the lag time of protein release can nearly correlate with the duration of the polyanhydride erosion shown in Fig. 2. For instance, the lag time prior to protein release is ca 98 h for the device using 20 mg of PSP (65:35) as isolating layers and the hydrolytic duration of 20 mg of the same polymer is ca 115 h. In addition, the pulse duration is also comparable with the protein release period from the PMAA/PEOx complex shown in Fig. 4, e.g. the pulse duration is ca 40 h from the device using 20 mg of PMAA/PEOx complex and the complete release period is 46 h from 20 mg of the same coated complex. Such correlation can be further demonstrated in

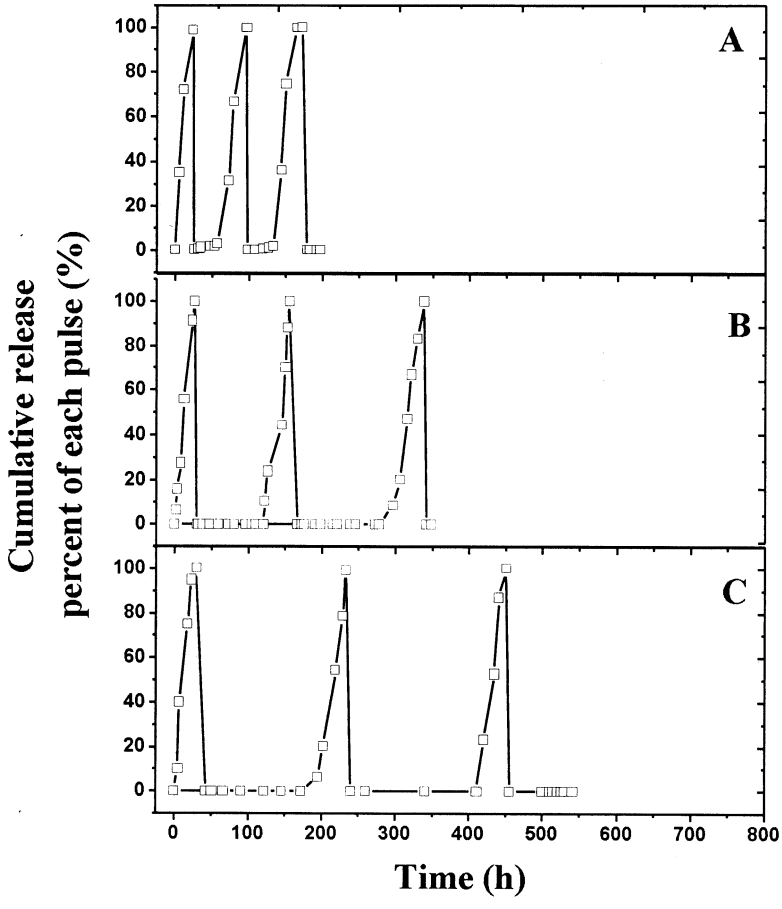

Fig. 6. Pulsed release profiles of proteins from the laminated device compressed under the conditions described as experiment section. Each protein-loaded layer was compressed with 20 mg of complexes. (A) 20 mg of PTSP (50:30:20) as isolating layers with 2.2 mm in thickness (FITC-BSA as model protein); (B) 20 mg of PSP (65:35) as isolating layers with 2.2 mm in thickness (Mb as model protein); (C) 30 mg of PSP (65:35) as isolating layers with 3.2 mm in thickness (Mb as model protein) ($n = 3$).

Table 2
Device description

Isolating layer	Type	PSP (65:35)	PSP (65:35)	PTSP	PSP (65:35)
	Weight (mg)	20	20	20	20
Drug-loaded layer	Type	PMAAa/PEOx	PMAA/PEOx	PMAA/PEOx	PEG[a]
	Weight (mg)	20	20	20	20
Model drug		Mb	FITC-dextran	FITC-BSA	Mb
Device size (ϕ mm × mm)		2.8 × 13.5	2.8 × 13.6	2.8 × 13.6	2.8 × 13.4
Device designation		I	II	III	IV

[a] Polyethylene glycol, $M_w = 6000$.

Fig. 7, which shows PTSP erosion rate, BSA release rate and pH variation with time from the device III. It can be seen that the lag time of BSA release equals the erosion duration of PTSP. In addition, the erosion of PTSP is nearly negligible during the period of BSA release, which can be attributed to the high hydrophobicity and surface-erosion characteristics of PMAA/PEOx complex, thus preventing the water ingress during BSA release period. The pH variation of the dissolution fluid is in accordance with the PTSP erosion. During the polymer erosion, pH changes from 3.8 to 5.2, which is similar to that shown in Fig. 3; while in the period of BSA release, the pH maintains at ca 7.4, which can attribute to the fact that the BSA release period from the laminated device is comparable to the release duration at pH 7.4 shown in Fig. 4.

From the results shown in Figs. 3 and 7, it is reasonable to think that the inner pH in the laminated device maintains the value lower than 5.0 during erosion of the polyanhydride contacting with the dissolution fluid. The low pH in the device stabilizes the inner pH-sensitive complexes and retains the incorporated proteins until the upper polyanhydride degrades completely, resulting in the pulsatile protein release. Such synergistic effect can be further confirmed by following results. It was found that the Mb release from the device IV displayed an irregular pattern, an initial burst within ca 5 h, a dormant period of about 30 h and a subsequent sustained release phase (lack of second dormant period). In addition, the release of FITC-dextran from the device II displays a similar trend with that of Mb release from the device IV. Considering the pH-insensitive release of Mb from PEG and dextran, such results can be attributed to the water penetration into the inner protein-loaded layer and the production of porous inner structure in the upper degrading polyanhydrides, which results the entrapped drugs diffuse out before the upper polymer degrades completely. High water content and porosity were in general observed in the degrading polyanhydrides (Göperferich and Langer, 1993; Mäder et al., 1997a). Therefore, the synergistic effect between

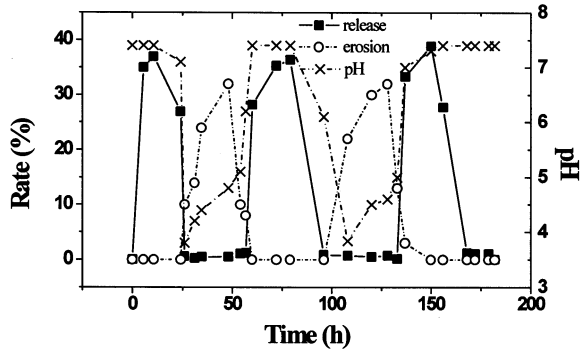

Fig. 7. Erosion rate of PTSP, BSA release rate and pH variation of the dissolution fluid for the device comprising of 20 mg of PTSP and 20 mg of PMAA/PEOx complex versus time. PTSP erosion rate was measured by the absorbance of degrading fragments with an UV–Vis spectrophotometer at 420 nm, FITC-BSA was detected with a fluorescence spectrophotometer and the pH of the dissolution fluid was obtained with a pH meter. The device was compressed under the condition described as experiment section ($n = 3$).

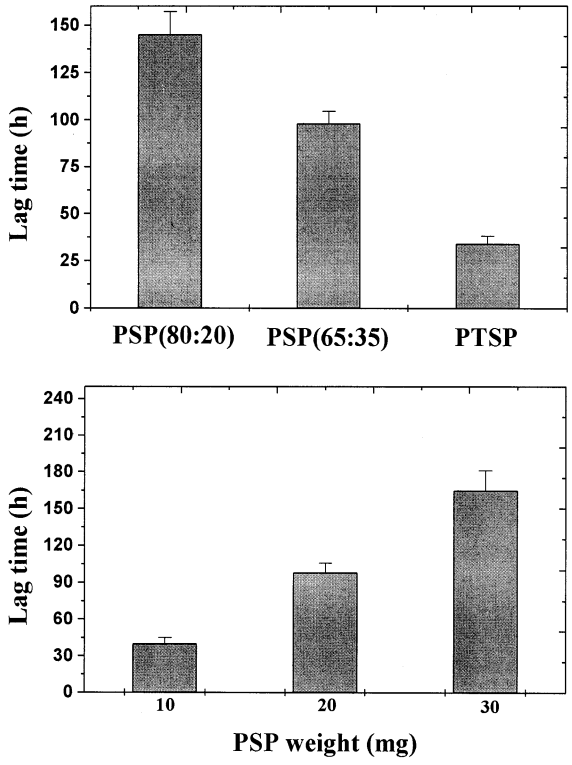

Fig. 8. Influence of (A) polyanhydride types (20 mg) and (B) mass of isolating layers (PSP (65:35)) on the averaged lag time. The device was compressed under the condition described as experiment section ($n = 3$).

the degrading polyanhydrides, the pH-sensitive complexes and proteins is necessary to maintain the protein release in a pulsatile manner from the laminated device.

Fig. 8 shows that the lag time of protein release can be modulated by the polyanhydride types and mass. The slower the polymer erodes, the thicker the layer is, and the longer the lag time is. Beside the polyanhydride structure and composition, the erosion duration of polyanhydrides also depends on their shape and mass (Tamada and Langer, 1993), which results in the increase in lag time with isolating layer mass. It can also be seen in Fig. 9 that the duration of each pulse depends on the complex mass, e.g. the protein release time of each pulse is about 40 h as 20 mg of PMAA/PEOx complex is used as protein-loaded layers, and 18 h for 10 mg of PMAA/PEOx complex, which is comparable to Mb release period shown in Fig. 5.

It was observed that ca 60% Mb denatured in form of precipitators when directly entrapped into the polyanhydrides, however, the Mb release amount from the laminated device is almost equal to that theoretically calculated, which indicates that by entrapping Mb into complex aggregation during polyanhydride erosion can be avoided. The soret band of Mb released from the device is identical with that of natural Mb, which indicates that the average heme environment in Mb is not altered upon entrapment in complexes and residence in acidic microclimate created by degradation of the polyanhydrides (Edmiston et al., 1994). It was reported that protein degradation in acidic environment could be prevented by maintaining the protein as a native insoluble aggregate or excipient complex (Johnson et al., 1996). In addition, irreversible aggregation of proteins due to the hydrophobic interactions between hydrophobic polymers and the hydrophilic protein is thought to be one of the main factors contributing to the loss of activity (Cleland and Langer, 1994). Therefore, entrapment of proteins into the pH-sensitive complexes is superior to the direct incorporation of proteins into hydrophobic polyanhydrides in maintaining the protein stability.

Although this is a preliminary work model, the concept that combines polyanhydrides and pH-sensitive complexes by the synergistic effect to

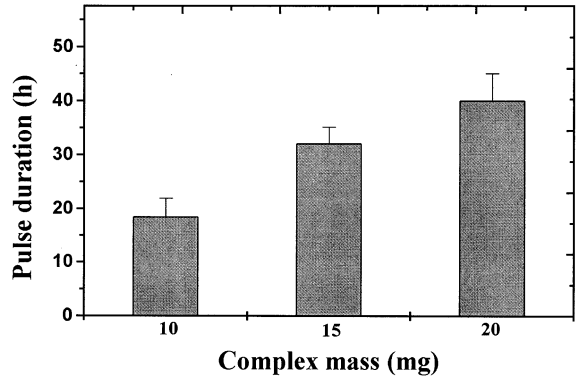

Fig. 9. Influence of mass of PMAA/PEOx complex on pulse duration. The device was compressed under the condition described as experiment section ($n = 3$).

finely modulate protein pulsed release dynamics and maintain protein stability may be useful for a single-shot vaccine or other related applications.

4. Conclusions

A laminated device composed of polyanhydrides as isolating layers and pH-sensitive complexes as protein-loaded layers was proposed to deliver proteins in a pulsatile manner. The protein pulsed release pattern, i.e. both lags time in protein release and release duration of each pulse, can be modulated by varying polyanhydride layer mass, types and complex mass, respectively. In addition, the model proteins release in a controllable fashion that is determined by the duration of polyanhydride erosion and complex dissolution, which makes it easy to finely tailor the protein pulsed release behavior. The results can be attributed to the synergistic effect between polyanhydride, the pH-sensitive complexes and proteins during polyanhydride erosion.

Acknowledgements

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