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Research Article

Controlled Delivery Systems for Proteins Based on Poly(Lactic/Glycolic Acid) Microspheres

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This paper describes an investigation of the use of poly(lactic/glycolic acid) polymers for long-term delivery of high molecular weight, water-soluble proteins. Poly(lactic/glycolic acid) (PLGA) microspheres, containing (fluorescein isothiocyanate)-labeled bovine serum albumin and (fluorescein isothiocyanate)-labeled horseradish peroxidase, were prepared by a modified solvent evaporation method using a double emulsion. The microspheres were spherical with diameters of 55–95 μm and encapsulated more than 90% of the protein. The preparation method was gentle and maintained enzyme activity and protein solubility. Stability studies showed that the encapsulation of an enzyme inside PLGA microspheres can protect them from activity loss. When not placed inside PLGA microspheres, (fluorescein isothiocyanate)-labeled horseradish peroxidase lost 80% of its activity in solution at 37°C in a few days, whereas inside the PLGA microspheres it retained more than 55% of its activity after 21 days of incubation at 37°C. *In vitro* release studies revealed that different release profiles (i.e., near-constant or biphasic) and release rates can be achieved by simply modifying factors in the preparation procedure such as mixing rate and volume of inner water and organic phases. Degradation studies by scanning electron microscopy and gel-permeation chromatography suggested that the mechanism responsible for protein release is mainly through matrix erosion.

KEY WORDS: protein delivery system; controlled release; enzyme stability; poly(lactic/glycolic acid); biodegradable microspheres.

INTRODUCTION

Protein delivery has become an important area of research as a large number of recombinant proteins are now being investigated for therapeutic applications. However, some proteins have very short *in vivo* half-lives and, as a consequence, multiple injections are required in order to achieve desirable therapy. One way to increase the therapeutic efficiency of these polypeptides is encapsulating them in a sustained-dosage form that is capable of releasing the macromolecule continuously, at a controlled rate, over a period of weeks or even months.

We are investigating the feasibility of using poly(lactic/glycolic acid) polymers for the long-term delivery of high molecular weight proteins. These polymers have been used for many years as surgical sutures (1). The long experience with these polymers has shown that these materials are biocompatible in physiological environments and degrade to toxicologically acceptable products that are eventually eliminated from the body (2,3). The physical characteristics of poly(lactic/glycolic acid) (PLGA) include strength, hydrophobicity, and pliability. All these features make PLGA desirable for use in drug delivery applications.

Drug-delivery research with PLGA polymers has been largely confined to low molecular weight steroids for contraception (4–6) and to small peptides such as LHRH analogues (7–9). However, proteins differ from steroids or small peptides in their size, shape, and solubility. It is known that, in the absence of specific chemical interactions, polypeptides will be insoluble in polymers such as PLGA (10). Low or negligible solubility of the macromolecular drug in a polymer will prevent diffusional transport of the agent through the polymer phase. In this case, polymer degradation will play an important role in the mechanism of the protein release. Based on this understanding, we have searched for PLGA polymers with degradative properties that will allow the controlled release of high molecular weight, water-soluble proteins for a period of 4–6 weeks.

PLGA microspheres (copolymer ratio of 75/25 and molecular weights of 14,000 and less) were prepared by a modified solvent evaporation method. The effect of microencapsulation on protein solubility and enzyme activity was studied by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and bioassays, respectively. Their degradation *in vitro* was studied by gel-permeation chromatography and scanning electron microscopy and was correlated with the observed protein release.

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MATERIALS AND METHODS

Materials

PLGAs with a copolymer ratio of 75/25 [lactic/glycolic (%)] and MWs of 5,000, 10,000, and 14,000 daltons were from Wako Pure Chemical Ind. Polyvinylalcohol (PVA) of average MWs 77,000–79,000, 88% hydrolyzed, was from Aldrich Chemical Co. Fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) and FITC-labeled horseradish peroxidase (FITC-HRP) were from Sigma Chemical Co. Other materials were reagent grade.

Microsphere Preparation

PLGA microspheres were prepared by a modified solvent evaporation method using a double emulsion (5). Briefly, 10 mg protein was dissolved in 50 μl double-distilled water and poured into 1 g PLGA dissolved in 1 ml methylene chloride. The mixture was mixed for 1 min using a vortex mixer at maximum speed (Vortex Genie, Scientific Inc.), or probe sonicated (Model VC-250, Sonic & Materials Inc.) at output 4 (50 W), for 30 sec to form the first inner emulsion (W_1/O). The emulsion was poured, under vigorous mixing using a magnetic bar, into 2 ml of aqueous 1% PVA saturated with methylene chloride to form the second emulsion [$(W_1/O)W_2$]. The resulting double emulsion was poured into 200 ml 0.1% PVA and continuously stirred for 3 hr at room temperature until most of the methylene chloride evaporated, leaving solid microspheres. The microspheres were collected by centrifugation (Sorvall DoPont Model RC-5B, 1000*g* for 10 min), sized using sieves with apertures of 100 μm, and freeze-dried (16 hr, Freeze Dryer, Lab Conc) into a powder. Unless specified, studies were done with PLGA with a ratio of 75:25 (L/G) and number-average molecular weight of 10,000 daltons.

Microspheres composed of PLGA with a molecular weight of 5000 daltons were prepared with a slight modification; the second emulsion was prepared with 5 ml of 0.1% PVA and the evaporation step was conducted in 0.3% PVA.

Protein Recovery from PLGA Microspheres

The amount of protein (FITC-BSA or FITC-HRP) in PLGA microspheres was determined by two methods: directly by recovering the protein from PLGA microspheres and indirectly by measuring the residual unentrapped protein in the outer water phase. Three methods for protein extraction were studied: (1) microspheres were dissolved in 3 ml methylene chloride and the protein was extracted into 4 ml distilled water; (2) microspheres were dissolved in 2 ml 90% acetonitrile aqueous solution and FITC-BSA was extracted into 8 ml 0.1 *M* phosphate buffer, pH 7.4; and (3) microspheres were suspended in distilled water and bath-sonicated for 15 min. The aqueous solution was filtered through a 0.45-μm filter (Acro LC25, Gelman Sciences Inc.) and the amount of FITC-BSA and FITC-HRP was determined by their absorbance at 495 nm (Fast Scan 553, Perkin Elmer).

FITC-HRP Stability in PLGA Microspheres

FITC-HRP stability in solution and in PLGA microspheres was examined at 37°C as follows: 100 μg/ml of FITC-HRP in 0.033 *M* phosphate buffer, pH 7.2, with 0.02% Tween 80 and 30 mg of PLGA microspheres, at 1% loading FITC-HRP, in 4 ml of the same buffer were incubated at 37°C. Aliquots were taken at different time intervals and the enzyme activity was measured using 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) as a substrate.

The encapsulated FITC-HRP was extracted from PLGA microspheres as follows: microspheres were collected by centrifugation (1000*g*, 2 min), washed three times with distilled water to remove salts, and freeze-dried. The dry microspheres were resuspended in distilled water, where they swelled and released their contents. Microspheres were separated from the extracted protein by centrifugation (1000*g* for 10 min) and further filtered through a 0.45-μm filter (Acro LC25, Gelman Sciences Inc.). The amount of extracted FITC-HRP was determined by its absorbance at 495 nm and by a BCA protein assay (Pierce 23235).

SDS–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The integrity of FITC-BSA in PLGA microspheres was analyzed by SDS–polyacrylamide gel electrophoresis. FITC-BSA extraction was done as described in the previous section for FITC-HRP. The extracted protein was concentrated using a centrifugal concentrator (10,000 MW cutoff, Amicon) and was loaded on a PhastGel gradient 8-25 gels (Pharmacia). Separation and staining with silver nitrate were done with the PhastSystem (Pharmacia, File No. 110).

Phase-Contrast Light Microscopy

Microsphere shape and size were estimated using a light microscope (Micro Star, American Optical, Buffalo, NY). Samples of PLGA microspheres were suspended in aqueous solutions and placed on micro slides (Red Label, Thomas Scientific). Pictures were taken and the microsphere size distribution in a given field was analyzed according to a reference scale.

Release Studies

PLGA microspheres (30 mg) were suspended in 4 ml 0.033 *M* phosphate buffer, pH 7.2; 0.02% Tween 80 was added to prevent the microspheres from clumping. Release studies were conducted in a rotating 10-ml polypropylene bottle at 37°C in an air gravity incubator (Imperial Incubator, Lab Line Instruments Inc.). The samples were collected periodically, centrifuged for 10 min at 2000 rpm, filtered through a 0.8-μm filter (Syringe Filter, Nalge Company), and analyzed for the released FITC-BSA by monitoring its absorbance at 495 nm.

Gel-Permeation Chromatography (GPC)

Molecular weights of PLGA polymers before and after microsphere preparation and during degradation studies were measured on a Perkin–Elmer GPC system (Perkin–Elmer) consisting of the Series 10 pump and the 3600 data station with refractive index detection. Samples from degradation experiments were freeze-dried, redissolved in chloroform, and filtered through glass wool. The samples were eluted with chloroform through a PL-Gel column [Polymer

Laboratories, PL-Gel, poly(styrene-divinyl benzene), 30 cm × 1.5 mm, 5-μm particle, mixed bed] at a flow rate of 1 ml/min. The molecular weights were determined relative to polystyrene standards (Polysciences, Pennsylvania; molecular weight range of 2500–500,000) using CHROM 2 and GPC 5 computer programs (Perkin–Elmer).

Scanning Electron Microscopy (SEM)

Morphology and degradation of PLGA microspheres were studied on a scanning electron microscope (Cambridge Instruments, 250 Mk 3 or Amray AMR1000A) using 3–10 kV. Samples for SEM were freeze-dried, mounted on metal stubs with double-sided tape, and coated with gold to a thickness of 200–500 Å.

RESULTS

(FITC-BSA)-PLGA Microsphere Characteristics

Poly(lactic/glycolic acid) microspheres, prepared by the modified solvent evaporation method using double emulsion, were spherical (Fig. 1A). Size distribution measurements by light microscopy of several preparations showed that more than 90% of the microspheres had diameters in the range of 55–95 μm (Fig. 1B).

Microsphere size and performance depended on the mixing rate used in the preparation of the inner emulsion. Since PLGA solutions were transparent, it was possible to follow visually the microspheres during the different stages of preparation (before solidification). It was found that when the inner emulsion was prepared by vortex mixing, the resulting microspheres were larger with an extremely large inner emulsion. These microspheres encapsulated only 60% of the protein and released 70–80% during the first day of release experiments. When prepared by probe sonication, a microfine inner emulsion was formed and the overall microsphere size was much smaller. These microspheres encapsulated more than 90% of FITC-BSA and the initial release decreased significantly, to the range of 10–20% during day 1.

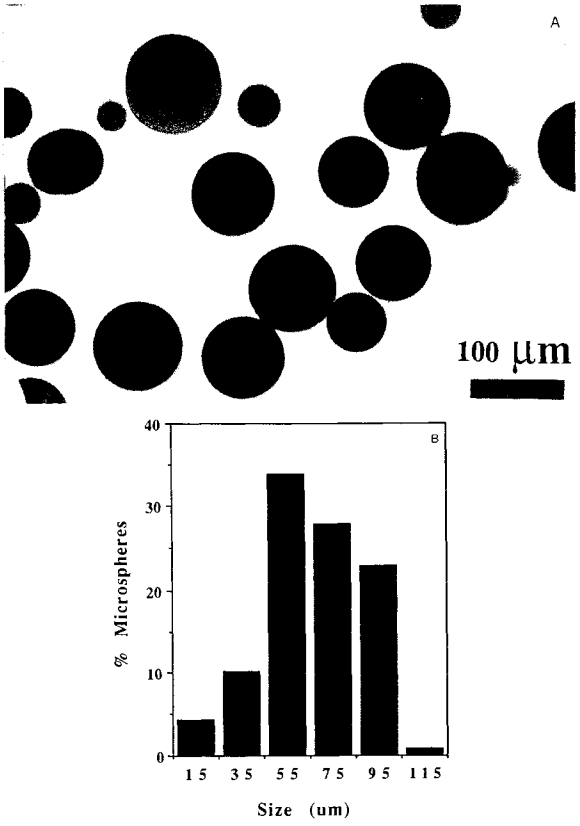

Fig. 1. (A) Micrograph of PLGA microspheres by phase contrast light microscopy. (B) Size distribution analysis of PLGA microspheres by light microscopy. Microspheres were prepared by the modified solvent evaporation method and sized with sieves of 100-μm aperture.

Stability of Proteins in PLGA Microspheres

Attempts to extract FITC-BSA from PLGA microspheres (without destroying the microspheres) immediately after they were prepared showed that negligible amounts of protein were recoverd. Only when microspheres were dissolved in methylene chloride was it possible to extract the protein into the aqueous phase. After 1 day in releasing medium, the amount of protein extracted from the intact microspheres increased; 7 days later it was possible to extract most of the protein. These results suggest that at early stages the protein is tightly held by the polymer matrix. With the degradation of the polymer, the matrix erodes and pores are formed (as seen by SEM, Figs. 6B–F (see below), allowing the extraction of the proteins.

SDS-PAGE analysis of the extracted protein at different times showed one band that correlates with intact FITC-BSA (Fig. 2). These results suggest that the protein does not interact chemically with the polymer matrix and that it is held physically by the dense polymer matrix. Moreover, they indicate that the method of FITC-BSA encapsulation in PLGA microspheres, which involves an organic solvent such as methylene chloride, is gentle and does not lead to a significant irreversible aggregation of the protein. This implication is supported by the results of release studies showing that more than 80% of FITC-BSA is released from PLGA microspheres (see below).

To study protein stability in PLGA further, FITC-HRP was encapsulated in microspheres and its activity was followed for 3 weeks. The results, depicted in Fig. 3, show that

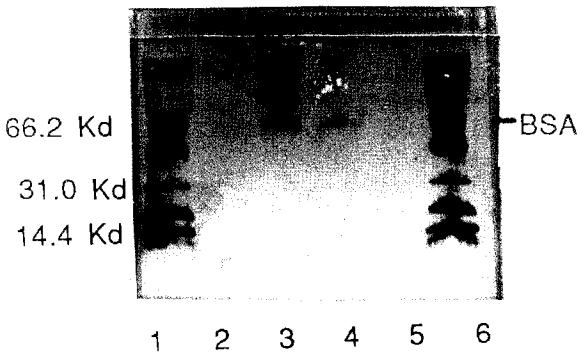

Fig. 2. SDS–polyacrylamide gel electrophoresis of FITC-BSA extracted from PLGA microspheres. Lane 1 and 6, protein standards; lane 3, protein extract after 7 days at 37°C; lane 4, protein extract after 14 days at 37°C.

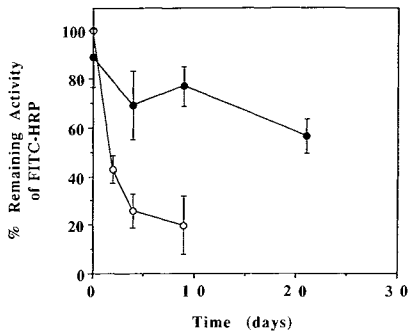

Fig. 3. Stability of FITC-HRP at 37°C in solution (○) and in PLGA microspheres (●).

the enzyme lost less than 18% of its activity following the microencapsulation process. However, FITC-HRP lost 80% of its activity in solution at 37°C in a few days, whereas inside the PLGA microspheres it retained more that 55% of its activity after 21 days. These studies indicate that the encapsulation of FITC-HRP in PLGA microspheres stabilized the enzyme.

In Vitro Degradation of PLGA Microspheres by Gel Permeation Chromatography

The degradation of PLGA in the presence of FITC-BSA was characterized in terms of molecular weight distribution using GPC. Figure 4 shows the GPC chromatograms of PLGA microspheres with a copolymer ratio of 75/25 lactic/glycolic and initial average molecular weight of 10,480 (±249) daltons at different stages of degradation. The solid line represents a typical molecular weight distribution of PLGA microspheres immediately after preparation; it is identical to the molecular weight distribution before device fabrication. The dashed lines are PLGA microspheres after 7 and 14 days in the immersion medium. On degradation, a distinct shift in the PLGA peak to higher elution times, corresponding to a progressive decrease in the modal molecular weight of PLGA polymers, is seen. The molecular weight distribution at all times was unimodal and relatively narrow, with no evidence of shoulders corresponding to low or intermediate molecular weight fragments. Figure 5 summarizes the GPC studies, showing that the molecular weight of PLGA decreases linearly with time. After 21 days in immersion medium the molecular weight of PLGA decreased from 10,480 to 2671 daltons. The finding that no oligomers or low molecular weight fragments are seen via GPC suggests that PLGA polymers degrade to water-soluble fragments that are not soluble in chloroform, the solvent used to elute the samples. Future studies will examine the use of NMR to detect and evaluate the nature of the degraded oligomers.

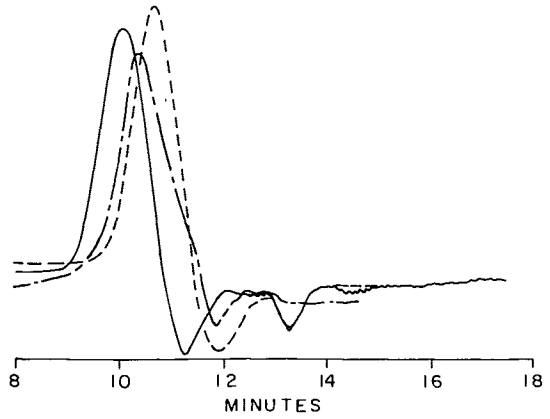

Fig. 4. GPC chromatograms of PLGA microspheres with a 75/25 lactic/glycolic acid ratio and an initial number-average molecular weight of 10,480 daltons, at different stages of degradation. The solid line is the molecular weight distribution of PLGA microspheres immediately after preparation; dashed lines are PLGA microspheres after 7 days (— - — - —) and 14 days (– – –) in releasing medium.

In Vitro Degradation of PLGA Microspheres by Scanning Electron Microscopy

Figure 6A is a typical scanning electron micrograph of PLGA microspheres prepared from PLGA with a molecular weight of 10,000 and a 75/25 lactic/glycolic acid ratio. Immediately after preparation, the microspheres show an intact outer surface; close examination reveals small micropores (diameter less that 0.1 μm) scattered all over the microsphere surface. Figures 6B–F display the PLGA microspheres at different stages of degradation. After 1 day in releasing medium, very small pores are seen. The microspheres are slightly crenated, suggesting that the microspheres adsorbed water when immersed in the releasing medium and shrank upon dehydration for SEM studies. After 4 days in releasing medium, small pores are seen scattered all over the microspheres. The pore size increased with time, and by day 14 the microspheres were highly porous, although they maintained their spherical shape. Seventy-six days later, only porous remnants of microspheres remained.

In Vitro Release of FITC-BSA from PLGA Microspheres: Effect of PLGA Molecular Weight

Figure 7 shows the release profile of FITC-BSA from PLGA microspheres with 1% loading as a function of the initial polymer molecular weight. The release profile of all systems was characterized by a typical initial protein burst. It is believed that the protein burst is due to protein release from the microsphere surface. The extent of the protein burst decreases with increasing PLGA molecular weight from 5000 to 10,000 and 14,000 daltons. Microspheres of PLGA with 5000 daltons released almost 70% of the protein

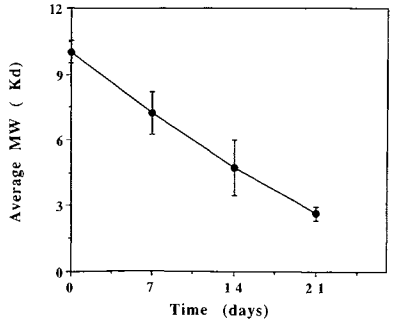

Fig. 5. Number-average molecular weight as a function of time, at 37°C.

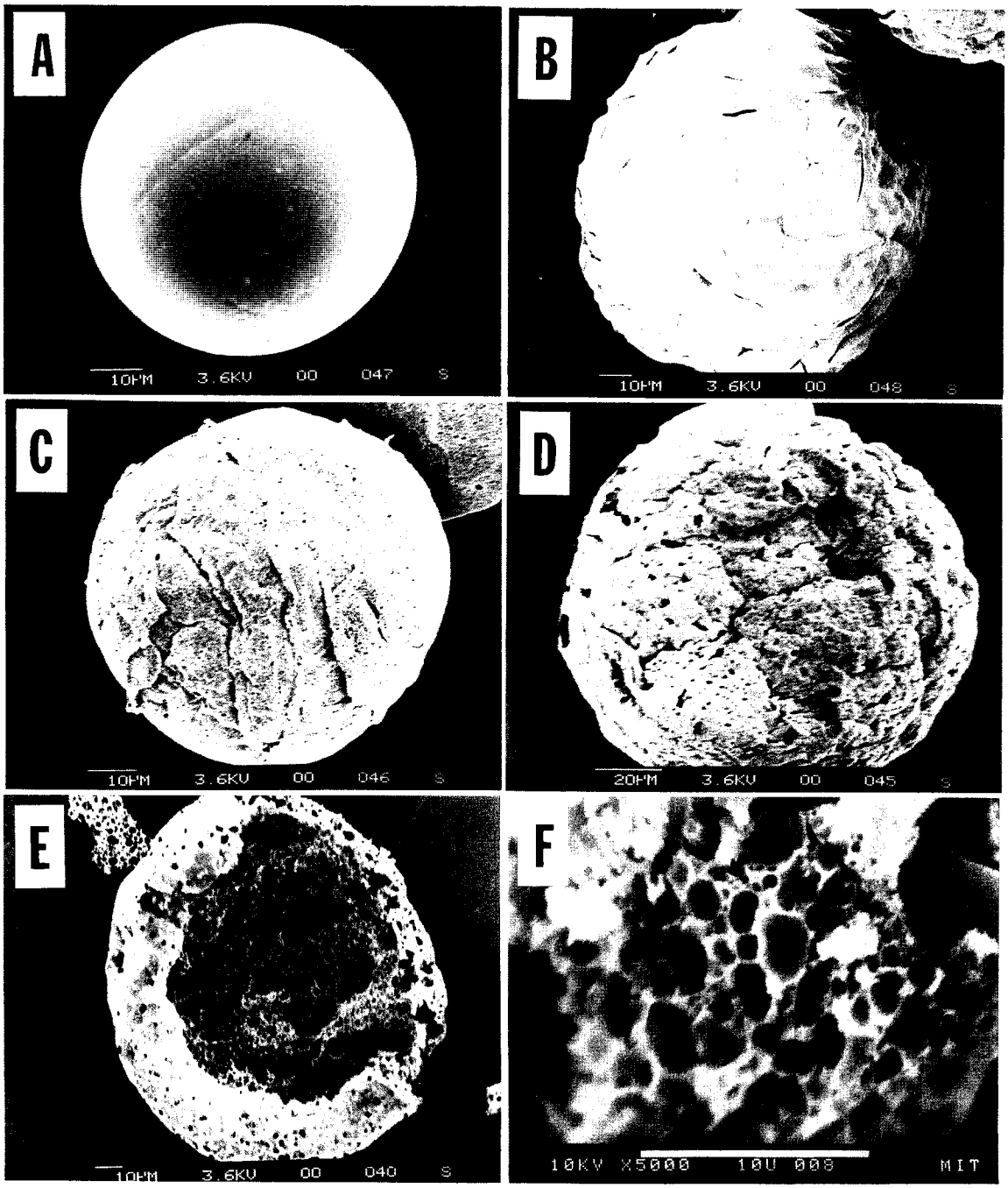

Fig. 6. SEM of PLGA (75:25 L/G) microspheres at different degradation states. Immediately after preparation (A); after 1 (B), 4 (C), 7 (D), 14 (E), and 76 (f) days in releasing medium at 37°C.

during the first day, while those composed of 10,000 and 14,000 daltons released less than 20%.

Visual examination of the 5000-dalton PLGA microspheres revealed that they are not spherical, and in some cases aggregates were observed. The percentage yield of microspheres was small, in the range of 50% (by weight), while with the 10,000 daltons PLGA, the yields were more than 90%. Thus, it seems that the 5000-dalton PLGA is not capable of forming stable microspheres by the modified solvent evaporation method.

Following the initial protein burst, a near-constant release is observed for almost 40 days, with no difference in the release rates from PLGA microspheres with polymer molecular weights of 10,000 and 14,000 daltons.

FITC-BSA Release from PLGA Microspheres: Effect of Inner Water and Organic Phase Volumes

The method for preparing PLGA microspheres is based on a double emulsion of water (W_1) in organic phase (O) in water (W_2), where the protein is dissolved in the inner water phase (W_1) and the polymer in the organic phase (i.e., meth-

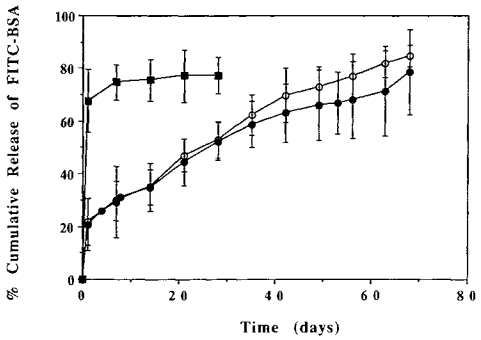

Fig. 7. *In vitro* cumulative FITC-BSA release from PLGA (75:25 L/G) microspheres as a function of polymer molecular weight: (■) 5000-, (●) 10,000-, and (○) 14,000-dalton PLGA.

ylene chloride). It was considered that changing parameters such as the volume of inner water or organic phase might affect the type of emulsion formed, thus affecting the overall protein release profiles.

Figure 8 shows the effect of inner water phase volume on the extent of initial protein burst. When FITC-BSA was added as a powder to the organic phase, more than 70% of the protein were released immediately upon immersion in the buffered medium. This is explained by the insolubility of FITC-BSA in methylene chloride, which can give rise to a nonuniform distribution of large protein islands in the polymer matrix, presumably closer to the microsphere surface, and thus result in their fast release upon immersion in the medium. Thus, in the case of water-soluble proteins, the double-emulsion method that combines water and organic phases is suitable for encapsulation. With the addition of inner water phase (30 μl) the initial protein burst decreased to 50% release. Increasing the inner water phase volume to 50 μl, while keeping the amount of added protein constant, resulted in a further decrease in the initial protein burst to less than 20% during the first day of the study. Above this volume, a slight increase in the initial protein burst is observed. This might reflect a situation where the aqueous phase is too large for the formation of a stable emulsion of water in the organic phase.

The effect of oil phase volume was also studied. In these experiments, the inner water phase volume and the amount of polymer were kept constant (50 μl and 1 g, respectively) and the methylene chloride volume was varied from 0.7 to 2 ml. The results, summarized in Fig. 9, show that decreasing the oil volume to a minimum value of 0.7 ml produced PLGA microspheres with less than 10% initial protein burst. Below this value, problems of polymer solubility were encountered. The decrease in oil phase volume gave a highly viscous polymer solution, resulting in PLGA microspheres with a dense core, thus decreasing the initial protein burst.

The oil phase volume also affected the overall release profile pattern. As seen in Fig. 10, with 0.7 ml methylene chloride a biphasic release of FITC-BSA was observed. After the initial protein burst, a lag time of more than 20 days was observed, and then again a sharp increase in protein release rates occurred. The extended lag time may be attributed to the difficulty of water to penetrate the highly dense polymer matrix, resulting in a slower rate of polymer erosion and very little release of protein.

DISCUSSION

PLGA polymers meet the requirements needed from a matrix for drug delivery applications including suitable mechanical properties, biodegradability, tissue compatibility, and ease of processing. We have investigated the use of these polymers to deliver high molecular weight proteins at a constant or biphasic release rate over several weeks.

As polypeptides have a high molecular weight and are water soluble, while PLGA is soluble only in the organic phase, we have searched for methods of matrix preparation that will combine both organic and water phases. Thus, the double-emulsion method (11) for preparation of PLGA microspheres was adopted. The resulting microspheres were spherical, with diameters of 55–95 μm, and efficiently encapsulated FITC-BSA. The process of encapsulation, which includes exposure of the proteins to methylene chloride and freeze-drying, had no major effect on the enzymatic activity of FITC-HRP or solubility of FITC-BSA as shown by the bioassay and SDS-PAGE studies, respectively. Moreover, the encapsulation of FITC-HRP inside PLGA microspheres enabled it to retain its activity for a prolonged time. These results are encouraging since they imply that the process of encapsulation in PLGA is gentle for proteins. In the case of therapeutic proteins, additional studies to assure physicochemical integrity of the protein upon microencapsulation,

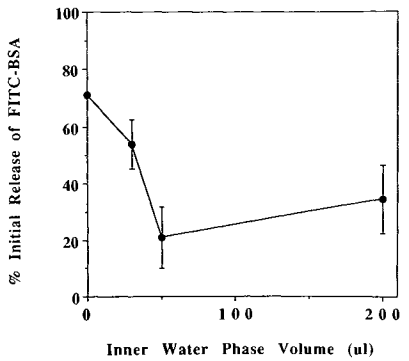

Fig. 8. The extent of initial protein burst as a function of inner water phase volume.

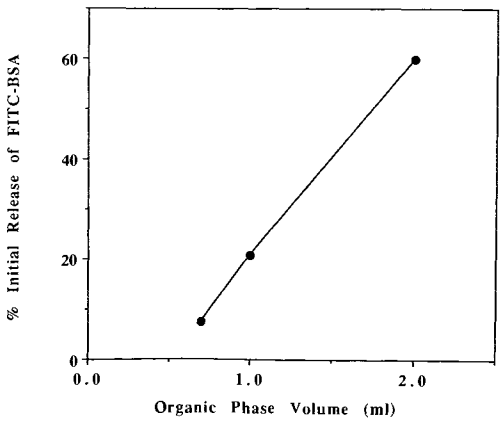

Fig. 9. The extent of initial protein burst as a function of organic phase volume (methylene chloride).

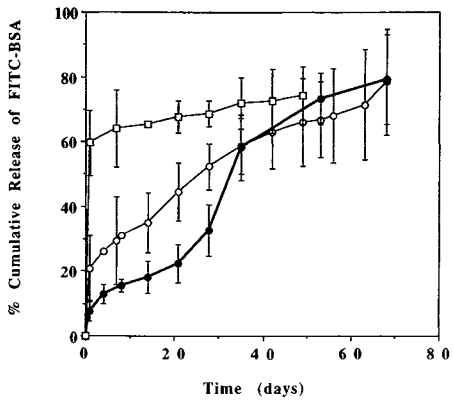

Fig. 10. *In vitro,* cumulative FITC-BSA release from PLGA (75:25 L/G) microspheres as a function of organic phase volume: (□) 2 ml, (○) 1 ml, and (●) 0.7 ml methylene chloride. The results are the average of two to five experiments.

and more importantly its biological activity will be necessary to satisfy the requirements of the regulatory authorities. Such studies include physicochemical characterization of the protein by gel-permeation chromatography, ultracentrifugation, and a variety of optical methods, including conformational analysis and biological assays (i.e., enzymatic activity or immunoassays) for potency or detection of any change in the biological activity of the protein upon microencapsulation. Since all or most of these methodologies are already applied in the preformulation stage of the therapeutic protein, it is considered that demonstrating the safety and efficacy of microspheres containing the protein will be relatively rapid.

Since most proteins are insoluble in PLGA, their release from these polyesters by classical partition-dependent diffusion is minimal. Consequently, the degradation of PLGA might be a critical factor in determining the release of proteins from these matrices. Thus, defining this parameter is the first step in the development of a time-specified, sustained-release dosage form. GPC studies showed that the degradation of PLGA *in vitro,* in buffer at pH 7.4, resulted in a progressive decrease in the molecular weight distribution of the polymer over time. The degradation under these conditions is not enzyme mediated and occurs by simple hydrolytic cleavage of ester groups. At all times, the molecular weight distribution displayed a unimodal pattern, suggesting a homogeneous degradation (at a constant rate) all over the matrix, implying that water penetrated throughout the polymer matrix. This result differs from other studies on PLGA degradation that showed the original polymer peak in addition to one or two shoulders at the low molecular weight tail of the GPC chromatogram (12,13). This may be due to our use of polymers with relatively lower molecular weights (10,000 and 14,000 daltons) than those used by other researchers (60,000 and more). Low molecular weight polymers are more hydrophilic, thus water uptake is faster and degradation occurs all over the matrix. The finding that no shoulders are seen at the low molecular weight tail of the GPC chromatogram implies that PLGA with initially low molecular weight degrades directly into water-soluble fragments.

Morphology studies by scanning electron microscopy supported a mechanism of homogeneous degradation of PLGA, showing a progressive formation of pores all over the PLGA matrix. Pores appear after the first day in the dissolution medium, and their size and number increased with time. By day 14, a highly porous but still spherical matrix is seen. After 76 days, only collapsed, porous pieces of microspheres are seen. These results correlate with the progressive loss in PLGAs molecular weight as seen by GPC.

Protein release from bioerodible polymer matrix can occur by two main routes: by diffusion through a tortuous, water-filled path in the polymer matrix and by matrix bioerosion (14,15). The latter occurs when the release of the protein from the matrix follows the erosion of the polymer surface and/or bulk matrix rather than by simple diffusion. Since these studies were conducted with PLGA microspheres at a relatively low protein loading (1% by weight), protein release through preformed, interconnecting pores was minimal. The near-linear decrease in polymer molecular weight and the fast progression in pore formation agree with the finding of near-constant release of FITC-BSA from PLGA microspheres, suggesting that protein release in our systems is controlled mainly by matrix erosion. The erosion is rapid because of our use of polymers with a low molecular weight, which give a relatively hydrophilic matrix, thus enabling fast penetration of water throughout the microsphere. This leads to the homogeneous degradation of the polymer into water-soluble fragments that are removed from the matrix. With the reduction in polymer molecular weight, the matrix becomes more and more hydrophilic, allowing more water to penetrate, thereby enhancing the degradation of polymer and, thus, protein release.

This paper continues a long-standing effort on the part of our laboratory to develop polymer-based systems to deliver proteins continuously. Our research started with the development of the first biocompatible controlled-release systems for proteins which were based principally on diffusion through pores in the polymer matrix (14). The model polymers studied included ethylene–vinyl acetate copolymer and various hydrogels. Subsequently, we developed degradable polymer systems capable of principally erosion control [model polymers were polyanhydrides (16)]. In the current study, the goal of designing controlled-release systems for 4 to 6-week delivery of protein was achieved by selecting polymers from the PLGA family with suitable degradative properties. However, polymer properties are only one of many parameters that affect protein release kinetics and/or the mechanism of their release (diffusion or erosion controlled) (14,15). Other parameters include the fabrication method (to produce a highly dense or porous matrix) and percentage of protein loading (to control the amount of preformed pores). By judiciously selecting desired polymer properties and formulation parameters, optimal kinetics for particular applications should be able to be achieved.

ACKNOWLEDGMENT

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