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Research paper

Applicability of various amphiphilic polymers to the modification of protein release kinetics from biodegradable reservoir-type microspheres

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Abstract

The effects of various amphiphilic polymers on the kinetics of protein release from reservoir-type microspheres, prepared by a solid-in-oil-in-water emulsion-solvent evaporation method, were investigated. Bovine serum albumin (BSA), as a model protein, was firstly micronized through co-lyophilization with amphiphilic polymers, such as poly (ethylene glycol) (PEG), polyvinylpyrrolidone (PVP), and pluronic F68. This process was based on the aqueous phase separation of protein and amphiphilic polymer induced by freezing-condensation. Mixing of poly(lactic-co-glycolic acid) (PLGA) and poly(lactic acid) (PLA) (at a ratio of 4:6) in a methylene chloride solution provided a'polymer-alloy' structure, where the preformed solid BSA microparticles were selectively distributed in the inner PLGA-rich phase. The reservoir-type microspheres obtained through this process showed high entrapment efficiencies (more than 85%) and reduced initial burst releases (less than 10%). Although PVP did not modify the BSA release profile, PEG and pluronic F68 enhanced the BSA release, with no increase of the initial burst effect, responding to their loading percentage: 3% loading of PEG or pluronic F68 resulted in typical zero-order release kinetics. The abilities of these amphiphilic polymers to modify the protein release profile could be predicted from their partitioning characteristics in the polymer-alloys and in the methylene chloride/water system.

Keywords: Reservoir-type microsphere; Poly(lactic-co-glycolic acid); Poly(lactic acid); Phase separation; Amphiphilic polymer; Poly(ethylene glycol); Polyvinylpyrrolidone; Release modification

1. Introduction

With recent developments in biotechnology and genetic engineering, many bioactive proteins are now available as therapeutic drug candidates [1,2]. In pharmaceutical research, the need for a method of administration other than conventional injection for these protein drugs has been growing. Microspheres made using biodegradable poly(lactic-co-glycolic acid) (PLGA) or poly(lactic acid) (PLA) for long-term release are being considered as a drug delivery system because of their potential convenience [3]. In fact, studies on the microencapsulation of human growth hormone [4], interferon [5] or erythropoietin [6] have already been reported.

Over the past two decades, there have been many reports concerning protein-loaded microspheres [5–9]. The preparation methods adopted in those studies can be basically classified into two types: a water-in-oil-in-water (W/O/W) emulsion method and a solid-in-oil-in-water (S/O/W) emulsion method [10]. Although the former method has the advantage of wide applicability, it has the problem that unstable protein molecules are easily denatured at the W/O interface [11]. On the other hand, the latter method is excellent in terms of protein integrity because solid-state proteins are stable in an organic solvent [10]. However, in order to efficiently entrap protein into microspheres by the S/O/W emulsion method, some micronization treatment of the protein material is required.

Recently, we have developed a new technology, as an alternative to the conventional spray-drying method, for obtaining spherical protein microparticles, which involves the lyophilization of a protein-poly(ethylene glycol) (PEG) aqueous mixture followed by the selective removal of PEG [12]. This method is based on the aqueous phase separation induced by PEG during freeze-drying, which provides a solid emulsion comprised of spherical protein microparticles dispersed in a continuous PEG phase. Because PEG can be selectively dissolved into an appropriate organic solvent due to its amphiphilic nature, a suspension of solid protein microparticles can be easily obtained. The mechanism of this tech-

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nology suggests that many amphiphilic substances other than PEG may have medical use as micronization adjuvants.

Our primary purpose in this study is to verify the applicability of various kinds of amphiphilic polymers (polyvinylpyrrolidone, pluronic and so on) to the above-mentioned protein micronization technology, using bovine serum albumin (BSA) as a model protein.

Generally, zero-order release kinetics are desirable for long-term releasing formulations, so that the plasma drug level reflecting pharmacological effects can be maintained. However, most protein-loaded microspheres show a triphasic release kinetics: an initial burst release, a lag-time, and a subsequent steady release [13]. In particular, the problem of the initial burst release, which may have serious side effects, is still to be solved. In this sense, the recent report of a 'polymer-alloys' method offering reservoir-type microspheres is noteworthy [14]. This methodology is based on the selective localization of hydrophilic drug particles in a phase-separated structure between PLGA and PLA, which can minimize the initial burst release of the drug. Therefore, the combination of this polymer alloys method and our previous protein micronization technology has the potential to provide a solution to the initial burst problem of protein-loaded microspheres [15].

Much research has focused on modifying the protein release using surfactants incorporated into microspheres [16–18]. However, the effects of these adjuvants on protein release from reservoir-type microspheres have not been studied.

Therefore, the second objective of this study is to investigate the applicability of various amphiphilic polymers, including PEG, to protein encapsulation and to the modification of the kinetics of BSA release from reservoir-type microspheres.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO). Poly(DL-lactic acid) (PLA) with a molecular weight (MW) of 20 000 (PLA0020), poly(DL-lactic-co-glycolic acid) (PLGA) of 50:50 lactic to glycolic acid copolymer ratio with a MW of 20 000 (PLGA5020) and poly(ethylene glycol) (PEG) with MWs of 4000 (4K), 6000 (6K) and 70 000 (70K) were obtained from Wako Pure Chemical (Osaka, Japan). L-PLA with a MW of 110 000 (L-PLA00110) was purchased from Boehringer Ingeheim (Ingeheim, Germany). Polyvinylpyrrolidone (PVP #30) and Pluronic F68 were supplied by BASF (Ludwigshafen, Germany) and Asahi Denka Kogyo (Tokyo, Japan), respectively. Dimethyl-β-cyclodextrin (DMβCyD) was purchased from Nakalai tesque (Kyoto, Japan). Micro-BCA protein assay reagent was obtained from PIERCE Chemical (Rockford, IL). All other reagents used were of reagent grade.

2.2. Micronization of BSA

The micronization experiments for BSA using various kinds of amphiphilic polymers were conducted as described in our previous study using PEG [12]. Briefly, a series of solutions containing a constant amount of BSA (4 mg) and an amount (0–36 mg) of amphiphilic polymer, prepared in glass test tubes, were frozen on a pre-cooled shelf at 50°C in a freeze dryer (RLE-52E5, Kyowa Vacuum, Japan), and then freeze-dried, as previously described. The solid was first dispersed in methylene chloride to dissolve the amphiphilic polymer, and then immersed in ethanol to determine the size distribution of the BSA particles with a laser light scattering particle size analyzer (SALD-1100, Shimadzu Co. Ltd., Japan).

2.3. Preparation of reservoir-type microspheres

The entire procedure for preparing BSA-loaded reservoir-type microspheres is schematically represented in Fig. 1. At first, 25 mg of BSA was dissolved with an amount of amphiphilic polymer (10–25 mg) in 1 ml of distilled water. This

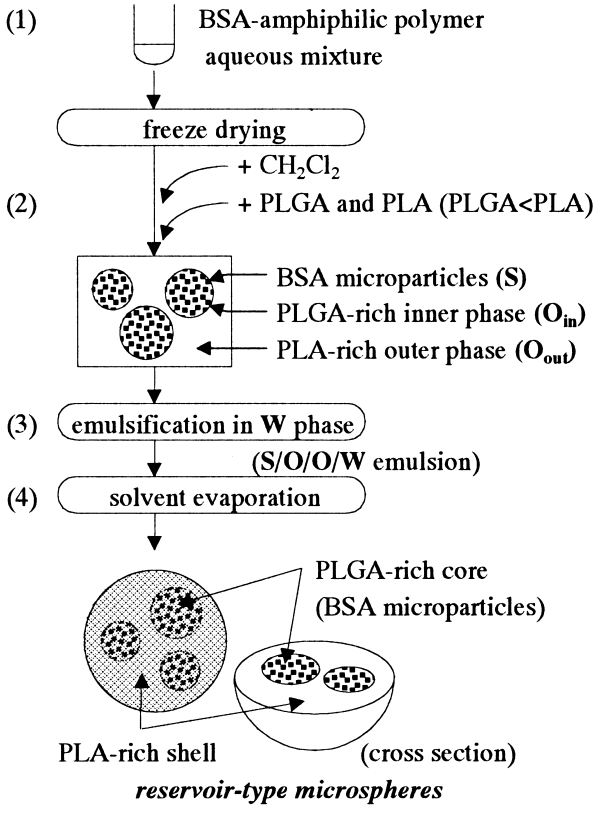

Fig. 1. A schematic representation of the procedure for preparing reservoir-type microspheres. The process comprises the following four steps: (1) protein micronization, (2) preparation of the organic phase, (3) emulsification for the preparation of the S/O/O/W emulsion, and (4) solvent evaporation. See section 2.2. for a more detailed explanation.

was followed by lyophilization as described in section 2.2. The obtained lyophilizate was dispersed in 1850 mg of methylene chloride, which resulted in a suspension of BSA microparticles (step 1). Then, prescribed amounts of PLGA, PLA, and L-PLA were added to the suspension to complete an organic phase (step 2). The total amount of solid materials was 500 mg. The mixing ratio of the three kinds of polymers (PLGA5020:PLA0020:L-PLA00110) was 40:54:6, which resulted in a polymer-alloy (phase-separated) structure comprised of an inner PLGA-rich phase and an outer PLA-rich phase in the organic phase [14]. The BSA microparticles were spontaneously localized in the inner PLGA-rich phase. This organic phase was added to 4 ml of a methylcellulose solution (0.25% w/v) maintained at 15°C, and homogenized with a polytron homogenizer (Kinematica Ag Littau, Switzerland) for 5 min at 8000 rev./min (step 3). The resulting S/O/O/W emulsion was quickly poured into 400 ml of distilled water, and then the temperature of this emulsion was raised stepwise up to 30°C for 3 h under vigorous stirring using a propeller mixer at 400 rev./min. The hardened microspheres were collected with a 20-μm mesh filter, washed with an excess amount of distilled water, and finally freeze-dried.

2.4. Determination of the size distribution of microspheres

BSA-loaded microspheres were suspended in distilled water containing Tween®-20 (0.02% v/v) for size distribution analysis with SALD-1100.

2.5. Microscopic study

Freeze-dried samples of the BSA-amphiphilic polymer aqueous mixture were observed with a scanning electron microscope (SEM; S-2250N, Hitachi Ltd., Japan).

2.6. Determination of protein content in microspheres

The total protein content in the BSA-loaded microspheres was determined according to the method of Sah [19] with slight modification. Briefly, microspheres (10 mg) were incubated for 1 h in 750 μl of dimethylsulfoxide, and then for a further 1 h after the addition of 2150 μl of a 0.5% sodiumlaurylsulfate/0.2 N-NaOH solution at room temperature to be completely dissolved. After neutralization with hydrogen chloride, the protein concentration in this solution was measured by a micro-BCA protein assay. Results were presented as 'entrapment efficiency' values, which indicate the percentage of protein entrapped in the microspheres with respect to the total amount of BSA loaded in the process.

2.7. In-vitro protein release studies

Microspheres (50 mg) were placed in test tubes and suspended in 10 ml of phosphate buffered saline (pH 7.4) containing 0.02% sodium azide. Incubation was conducted by rotating the test tube at 25 rev./min in an air chamber thermostatted at 37°C. At predetermined intervals, each test tube was centrifuged at 2000 rev./min for 5 min, and 9 ml of supernatant was withdrawn. After the addition of 9 ml of fresh medium, the test tube was returned to the air chamber and continuously incubated. The supernatant was filtered through a 0.5-μm membrane filter, and assayed for protein release by the micro-BCA protein assay.

2.8. Study of co-solubility between biodegradable polymer and amphiphilic polymer

A mixture of a biodegradable polymer (PLA or PLGA) and an amphiphilic polymer (PEG, PVP, or pluronic F68) at a weight mixing ratio of one was completely dissolved in an excess amount of methylene chloride. The solvent was slowly evaporated at room temperature, and the polymer concentration was calculated when the phase separation was observed. Results were presented as 'critical polymer concentration' (CCp) values.

2.9. Determination of the partition coefficient of amphiphilic polymer

Amphiphilic polymer (20 mg) was dissolved in 5 ml of methylene chloride, then 5 ml of distilled water saturated with methylene chloride was added. After shaking for 1 h at room temperature, each solvent phase was separated by centrifugation for 15 min at 3000 rev./min. The polymer content partitioned in each phase was determined by dry weight. Results were presented as Log *P*-values defined by the following equation

$$LogP = \log((Cp_{(MC)})/(Cp_{(w)}))$$

where $CP_{(MC)}$ and $Cp_{(w)}$ mean the polymer concentration in methylene chloride phase and water phase, respectively.

3. Results

3.1. Characterization of the protein micronization by various amphiphilic polymers

The applicability of various amphiphilic polymers as adjuvants in the protein micronization was studied, using BSA as a model protein. PVP, pluronic F68, and DMβCyD were examined other than three different MWs of PEG (4 K, 6 K, and 70 K). A series of aqueous mixtures containing BSA and amphiphilic polymer were freeze-dried, then dispersed in methylene chloride. Average diameters of the resulting BSA particles were plotted against the weight mixing ratio of amphiphilic polymer with BSA in Fig. 2. In all cases, the average size of the BSA microparticles decreased with the increase of the mixing ratio. A significant change in diameter (a bending point), shown by an arrow, was recognized in each relationship, implying that the formation mechanism of particles would be different above and below this point. BSA microparticles less than 10 μm in diameter could be obtained above the mixing ratio

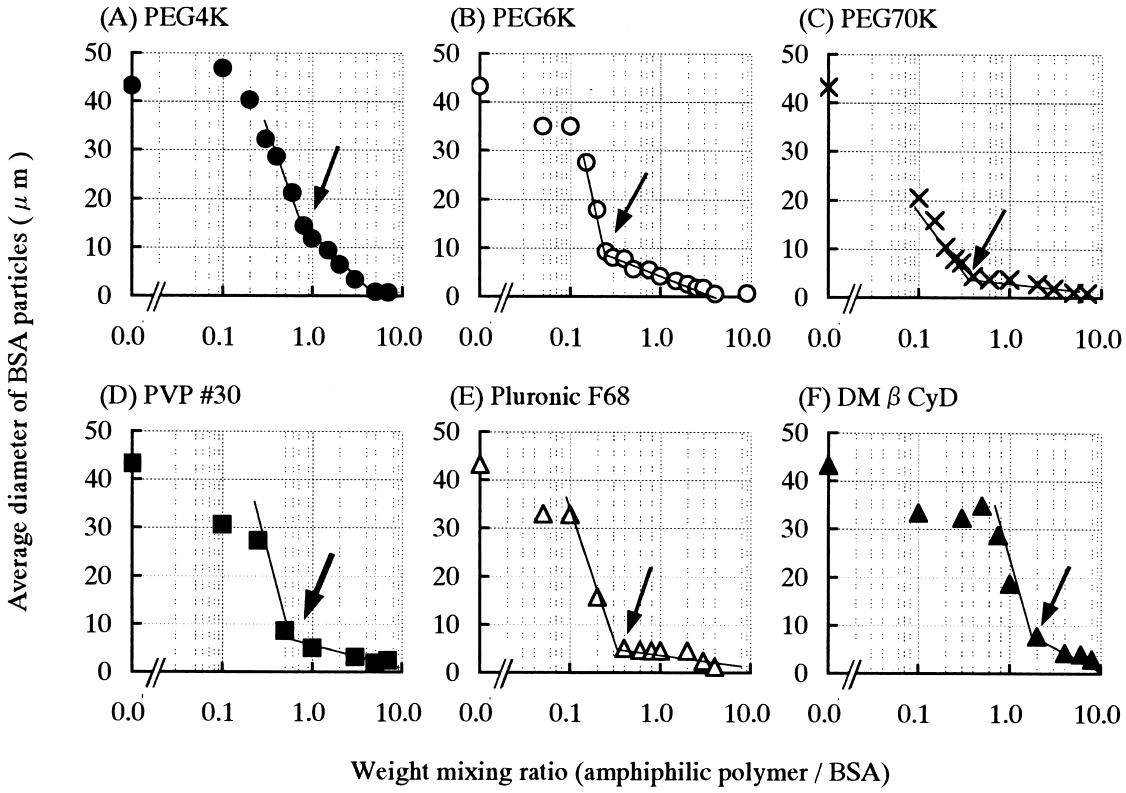

Fig. 2. The relationship between the weight mixing ratio of amphiphilic polymer with BSA and the average diameters of the dispersed BSA microparticles.

of this bending point. The mixing ratio providing the bending point was intrinsic for each amphiphilic polymer. That is, the bending point appeared at a mixing ratio of around 0.3 for PEG6K (Fig. 2B), PEG70K (Fig. 2C), and pluronic F68 (Fig. 2E), around 0.5 for PVP (Fig. 2D), and around 1 for PEG4K (Fig. 2A) and DMβCyD (Fig. 2F).

Fig. 3 shows typical SEM images of the co-lyophilizates of BSA with PEG6K (Fig. 3A), PVP (Fig. 3B), or pluronic F68 (Fig. 3C) at a mixing ratio of 1, and the colyophilizate of BSA with DMβCyD at a mixing ratio of 4 (Fig. 3D). These mixing conditions are all above the bending point, and spherical microdomains with an apparent size of 1–5 μm were observed in all cases. Therefore, by means of co-lyophilization with these amphiphilic polymers, spherical protein microparticles could be obtained in a particular range of conditions.

3.2. Application to the preparation of protein-loaded microspheres

3.2.1. Protein encapsulation into reservoir-type microspheres

The protein micronization treatment using various amphiphilic polymers was applied to the preparation of reservoir-type microspheres (Fig. 1), to examine the effects of these adjuvants on the entrapment efficiencies of BSA. BSA (25 mg) was dissolved in distilled water with 10–25 mg of PEG6K, PEG70K, pluronic F68 or PVP, which corresponds to 2–5% loading. This was followed by lyophilization. Reservoir-type microspheres were then prepared via a S/O/O/W emulsion. In Fig. 4, the entrapment efficiencies of BSA are shown in relation to the loading percentage of PEG6K or PVP. When using PEG6K, higher loading percentages of PEG6K resulted in slightly lower entrapment efficiencies, which were still higher than 85%. By contrast, in the case of PVP, the entrapment efficiencies of BSA declined with a decrease of the PVP loading, but were still higher than 90% at PVP loading percentages of more than 3%. However, the entrapment efficiency of microspheres made with 2% PVP loading dropped significantly to about 55%. Although the data are not shown in this figure, entrapment efficiencies higher than 85% were attained by the 3% loading of pluronic F68 or PEG70K.

3.2.2. In vitro release of BSA from the reservoir-type microspheres

Fig. 5 shows the in vitro dissolution profiles of these microspheres. In order to evaluate the effects of amphiphilic polymer on the BSA release, a release profile from microspheres without any amphiphilic polymer, prepared with BSA fine microparticles (purity $> 99\%$), was also obtained (closed circle). The initial burst (percentage released in the first 1 h) of the reference microspheres was very small (4.2%), which might result from the reservoir-type morphology achieved with the polymer-alloys method. After that, however, a very depressed release continued for at least 28

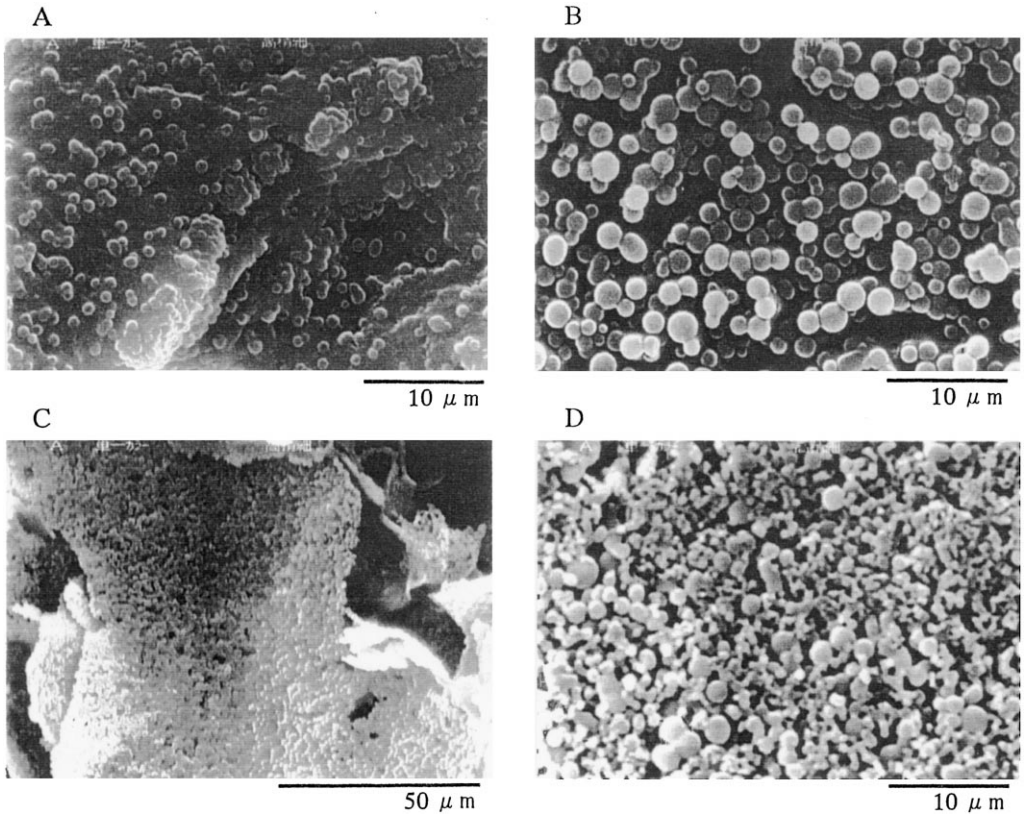

Fig. 3. SEM photographs of co-lyophilizates of BSA with various amphiphilic polymers. (A) PEG6K at the mixing ratio of 1. (B) PVP at the mixing ratio of 1. (C) pluronic F68 at the mixing ratio of 1. (D) DMβCyD at the mixing ratio of 4.

days. In contrast to this profile, the effects of amphiphilic adjuvants on the release kinetics of BSA differed. Although the initial burst release of BSA from the microspheres loaded with PEG6K was kept below 5% at all loading percentages studied, high loading percentages promoted the subsequent release of BSA (days 1–21). Particularly, the microspheres prepared with 3% loading of PEG6K showed almost constant release kinetics for about 3 weeks. Similar profiles were obtained by 3% loading of PEG70K (Fig. 5B) or 3% loading of pluronic F68 (Fig. 5D). On the other hand, the loading of PVP from 2–5% didn't have any effect on the BSA release except for a slight increase of the initial burst to about 10–15% (Fig. 5C).

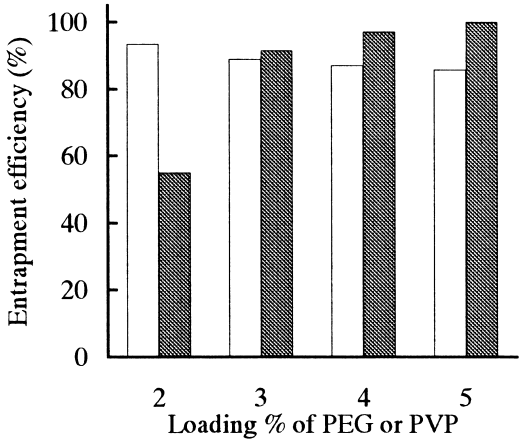

Fig. 4. The entrapment etliciencies of BSA with respect to the loading percentage of PEG6K or PVP. The open bar shows the entrapment efficiencies when PEG6K was loaded, and shaded bar shows the entrapment efficiencies when PVP was loaded.

In Fig. 6, typical SEM images of microspheres prepared with PEG6K or PVP are shown. Pores were recognized on the surface of the microspheres loaded with 5% PEG6K (Fig. 6A) or 5% PVP (Fig. 6B). However, the microspheres loaded with 3% PEG6K (Fig. 6C) or 3% PVP (Fig. 6D) possessed apparently smooth surfaces. The final diameter of these microspheres was about 37 μm for PEG-loaded microspheres and 32 μm for PVP-loaded microspheres, independent of their loading percentages.

4. Discussion

4.1. Mechanistic consideration of the protein micronization by amphiphilic polymers

Our previous paper introduced a new micronization technology for protein drugs including a freeze-drying process for a PEG-protein aqueous mixture [12]. Briefly, the principle of this method is based on the phase separation induced by freezing-condensation, and after the subsequent drying process, spherical microdomains of protein scattered

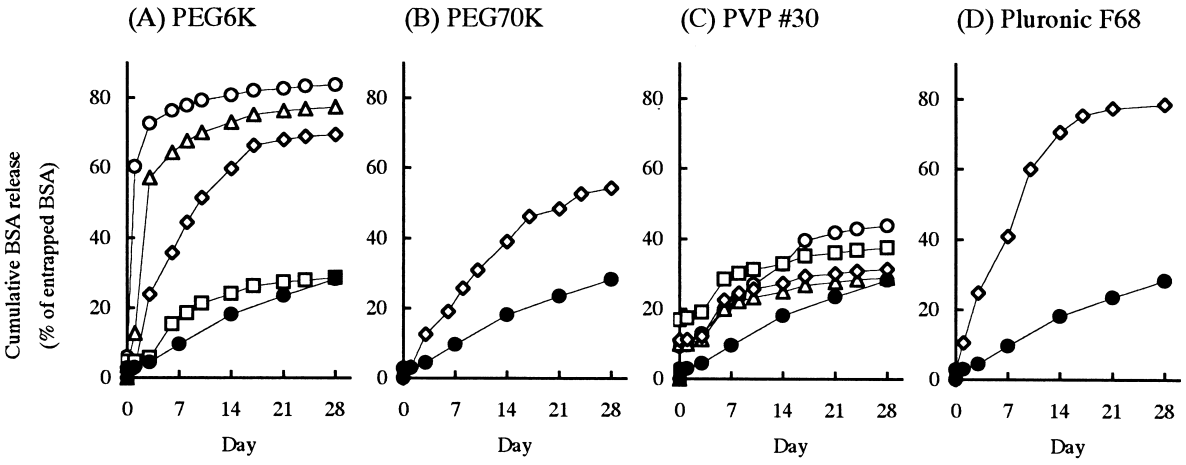

Fig. 5. In vitro release profiles of the BSA-loaded reservoir-type microspheres prepared with various kinds of amphiphilic polymer. The closed circle shows the release profile of the reference microspheres (see section 3.2.2.). The loading percentages of amphiphilic polymer are 2% (open square), 3% (open diamond), 4% (open triangle), and 5% (open circle).

in a continuous solid phase of PEG can be produced, in a particular condition range. Because PEG can be dissolved into methylene chloride, which is the most common solvent used for the preparation of microspheres, a suspension of micronized protein particles can be obtained.

In this study, using BSA as a model protein, various kinds of amphiphilic polymers were studied as an alternative micronization adjuvant. As shown in Figs. 2 and 3, spherical BSA microparticles less than 10 μm in diameter were obtained using all kinds of amphiphilic polymers studied. The size of protein microparticles can be tailored from the mixing ratio with these amphiphilic adjuvants. The significant change in diameter (a bending point) shown by an arrow in Fig. 2 indicates the point of 'phase transposition' of a water-in-water emulsion formed during the freezing process, as discussed in our previous paper [12]. Namely,

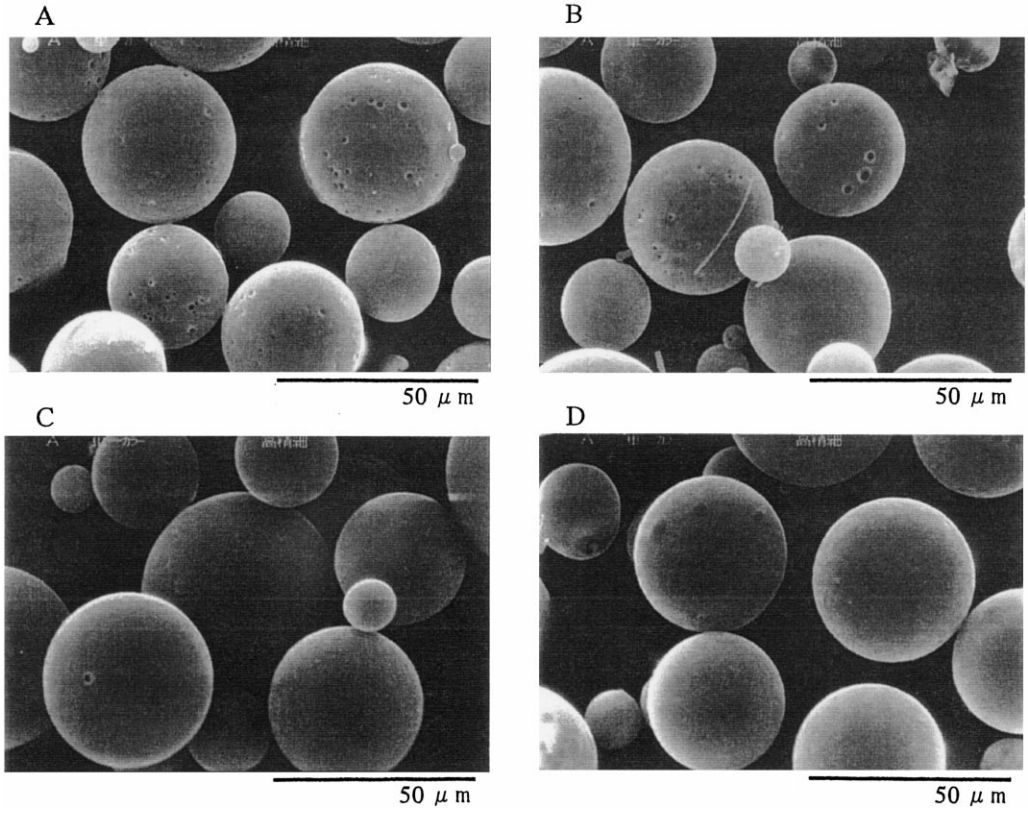

Fig. 6. SEM photographs of microspheres prepared with 5% loading of PEG6K (A), 5% loading of PVP (B), 3% loading of PEG6K (C), and 3% loading of PVP (D).

BSA forms a dispersed phase above this bending point, while amphiphilic polymer forms a dispersed phase below this point.

4.2. Protein encapsulation into reservoir-type microspheres

Matsumoto et al. [14] reported a procedure for preparing the reservoir-type microspheres by the polymer-alloys technique, in which pulverized *cis*-dichorodiammineplatinum was applied. This technology is based on the selective distribution of solid drug microparticles in a phase-separated structure between PLGA and PLA in methylene chloride, which depends on the interrelationship of the solubility parameters of PLGA, PLA and drug. Most hydrophilic compounds have a reportedly higher affinity to PLGA than PLA. Because the micronized BSA particles, in this study, were also spontaneously localized in the inner PLGA-rich phase, reservoir-type microspheres, as a result, could be obtained through a S/O/O/W emulsion process. Practically, an effective entrapment of more than 85% (Fig. 4) and suppressed initial burst of less than 5% (Fig. 5) are the advantages of the reservoir-type structure. The reason for the significantly low entrapment at 2% loading of PVP (Fig. 4) is the larger size of the BSA particles formed in the process of micronization. That is, the mixing ratio of 0.4 (PVP:BSA = 2%:5%) is smaller than the value of the bending point of the PVP/BSA system (around 0.5) seen in Fig. 2D. These results indicate that for the particles to be most effectively entrapped by the polymer-alloys method, they should be less than about 10 μm in size.

4.3. Effects of amphiphilic polymers on the in vitro release of BSA from reservoir-type microspheres

Protein-loaded microspheres often exhibit a tri-phasic release kinetics, being characterized by a phase of rapid release (due to surface located protein), a lag phase followed by a steady rate of release [13,20]. In the early phase including the lag phase, protein release is governed by diffusion-controlled mechanism through a network of water filled pores and channels. In the latter phase, erosion of the polymer matrices is considered to control protein release from the core of microspheres [21]. This general consideration should be applied to the reservoir-type microspheres in this study. So far, there have been several attempts to modify the diffusion-controlled release of protein by co-encapsulating non-ionic surfactants into PLGA microspheres [16–18]. For example, Cleek et al. reported that when 5% of PEG was loaded into an inner water phase by a W/O/W emulsion method, PEG content of PLGA microspheres was about 1.5%, which might accelerate the release of immunoglobulin by forming aqueous channels [16]. Blonco et al. [17] reported that the addition of poloxamer to PLGA microsphere formulations enhanced the BSA dissolution by inhibiting the interaction between BSA and polymer. However, the effect of these modifiers on protein release from reservoir-type microspheres has not been investigated.

In our study, the effects of PEG and PVP on the BSA release from the reservoir-type microspheres were quite different (Fig. 5). The influence of these amphiphilic polymers on the BSA release is discussed in terms of their behavior in each step of the microsphere preparation process.

Firstly, the localization of these amphiphilic polymers in a phase-separated organic phase should be considered. Table 1 shows the results of the co-solubility study using an amphiphilic polymer and a biodegradable polymer in a methylene chloride solution. Interestingly, all three kinds of amphiphilic polymers had higher CCp values with PLA than with PLGA. These results indicate that these amphiphilic polymers have higher affinity to PLA than PLGA in methylene chloride solution. This would suggest that these adjuvants exist predominantly in the outer PLA-rich phase rather than the inner PLGA-rich phase in the organic phase. Because the BSA microparticles coexist in the system, the possibility of some specific interaction between amphiphilic polymer and the BSA microparticles should be discussed. As explained in the previous section, in the microscopic observation of the organic phase, the BSA microparticles were dominantly localized in the inner PLGA-rich phase, whichever amphiphilic polymer was used. Therefore, the interaction between amphiphilic polymers and BSA microparticles seemed to be, if any, too weak to modify the distribution of BSA microparticles in the organic phase.

Secondly, in the emulsification and solvent evaporation process, these adjuvants have an opportunity to transfer from the organic phase to the outer water phase due to their amphiphilic nature. Thus, partition coefficients (Log *P*-values) of various amphiphilic polymers in a methylene chloride/water partitioning system were determined (Table 2).

PVP was more hydrophilic with a low Log *P*-value (−1.3), while all other amphiphilic polymers had high Log *P*-values (1–1.9).

Although the residual contents of these adjuvants in the final formulations were not determined in this study, the difference in their abilities to modify the protein release can be discussed as follows. The most definitive difference between PEG and PVP was the partition characteristics in the methylene chloride/water system (Table 2). Because PEG has a higher Log *P*-value in the methylene chloride/water system [22], it may be partly incorporated within microspheres. Further, the entrapped PEG may be distribu-

Table 1
The critical polymer concentration (CCp) inducing phase separation between amphiphilic polymers and biodegradable polymers in the methylene chloride solution

Amphiphilic polymer	PLGA (PLGA5020) %	PLA (PLA0020) %
PEG6K	12.2	51.9 < [a]
PVP#30	19.9	27.3
Pluronic F68	11.7	53.6 < [a]

[a] Phase separation was not observed.

Table 2
Partition coefficients (Log *P*-values) of various amphiphilic polymers in the methylene chloride/water partitioning system

Amphiphilic polymer	M W or grade	Log *P*
PEG4K	4000	1.88
PEG6K	6000	1.72
PEG70K	70 000	1.85
PVP	#30	− 1.39
Pluronic F68	8600	1.73
DMβ CyD	1200	1.09

ted in the PLA-rich shell of the reservoir-type microspheres (Table 1). Therefore, in the dissolution test, encapsulated PEG may facilitate the formation of aqueous pores through the PLA-rich shell, resulting in the enhancement of the diffusional release of BSA. Although the incorporation of PEG may alter the thermal properties of PLA-rich shell, the enhanced release in the early phase should be considered due to the increased diffusivity of BSA by the hydration of PEG.

A modification of the release characteristics by using different MWs of PEG may be possible. The BSA release from microspheres with 3% loading of PEG6K (Fig. 5A) was slightly faster than that from microspheres with 3% loading of PEG70K (Fig. 5B). This may result from the difference, between PEG70K and PEG6K, of their resolution rate and, in particular, the viscosity after the hydration in the microcircumstance of matrices. Because pluronic F68 has similar partitioning properties to PEG, the release kinetics of BSA can be modified. On the other hand, little PVP may be entrapped in the microspheres because most of it would leak into the outer water phase during the solvent evaporation process due to its hydrophilic nature. Thus, PVP has almost no effect on the BSA release.

The porous surface morphologies of the microspheres made with 5% loading of PEG or PVP (Fig. 6A,B) may result from the leakage of amphiphilic polymer during the solvent evaporation process. Despite these porous surface structures, the initial bursts of BSA were suppressed to less than 3% for PEG and 15% for PVP. This implies that the BSA microparticles would be firmly retained in the PLGA-rich core of the reservoir-type structure.

In this study, formulation experiments using DMβCyD or PEG4K were not carried out, because an excess of amphiphilic polymer was required in the micronization of BSA to obtain microparticles of the required size (less than 5 μm), as shown in Fig. 2. However, if the loading amount of protein or other formulation conditions were to be optimized, modifications of release by these adjuvants would be expected given their partitioning properties (Table 2).

5. Conclusion

Various amphiphilic polymers are applicable to our method of protein micronization through co-lyophilization. This micronization process could be combined with the preparation of reservoir-type microspheres by way of the S/O/O/W emulsion. PEG and pluronic F68 could enhance the protein release, and excellent zero-order release characteristics with reduced initial burst releases were demonstrated. The abilities of amphiphilic polymers to modify the protein release from the reservoir-type microspheres could be predicted from the partitioning characteristics in the polymer-alloys and in the methylene chloride/water system.

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