Influence of the degrees of hydrolyzation and polymerization of poly(vinylalcohol) on the preparation and properties of poly(DL-lactide-co-glycolide) nanoparticle

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Abstract

The influences of the degrees of hydrolyzation and polymerization of poly(vinylalcohol) (PVA) used as an emulsion stabilizer were investigated on the preparation of poly(DL-lactide-co-glycolide) (PLGA) nanoparticles with the spontaneous emulsification solvent diffusion process. The productivity of PLGA nanoparticles and the physical properties, i.e. particle size and redispersibility, of the resultant PLGA nanoparticles varied drastically depending on the PVA grade used. It was found that the degree of hydrolyzation of PVA was more important than the degree of polymerization to improve productivity and physical properties. The use of low-hydrolyzed PVA was useful to attain excellent productivity of PLGA nanoparticles, i.e. higher yield and uniform size distribution of nanoparticles, whereas highly hydrolyzed grade provided poor productivity and unsatisfied properties, e.g. poor redispersibility. A series of gelatinization studies of PVA suggested that the localized gelatinization of PVA preferentially occurred on the surface of emulsion droplets containing PLGA in the solvent diffusion process, thus influencing the formability of PLGA nanoparticles. These results indicate that proper choice of PVA grade was a key factor to determine the preparation behavior of PLGA nanoparticles. © 1997 Elsevier Science B.V.

Keywords: Poly(DL-lactide-co-glycolide); Nanoparticle; Poly(vinylalcohol); Hydrolyzation; Polymerization; Gelatinization

1. Introduction

During the past two decades, plenty of research has been conducted to apply biocompatible and biodegradable polymers to drug delivery systems. Among those polymers, much attention has been

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paid to poly(DL-lactide-co-glycolide) (PLGA) for the vehicle of microspheres because of its suitable physical properties for molding, processing, and controlling drug release. Meanwhile, colloidal drug carriers have been studied for various non-parenteral depot systems including oral (Eldridge et al., 1990; Ammoury et al., 1991); pulmonary (Gupta et al., 1990; Masinde and Hickey, 1993); nasal (Illum et al., 1988; Farraj et al., 1990); and ophthalmic (Vidmar et al., 1985; Losa et al., 1991) administrations, and lots of useful information has been reported so far (Jani et al., 1992; Lai et al., 1993). These findings seemed to extend the possibility of the development of a new colloidal drug delivery system.

In response to such recent progress in the basic research on the in vivo behaviors of colloidal particles, it should be desired to establish a new preparation concept of PLGA colloidal particles from the practical point of view. Recently, we found that the PLGA nanoparticles could be easily prepared by applying the spontaneous emulsification solvent diffusion method (SESD method) (Niwa et al., 1993), and it was suggested that poly(vinylalcohol) (PVA), an emulsion stabilizer, played an important role in the formation of PLGA colloidal particles and hence in the surface properties of the nanoparticles prepared.

Thus, the goal of our study was to establish a new technology for the preparation of colloidal nanoparticles. In this paper, the effects of types of PVA with various degrees of hydrolyzation and polymerization were investigated on the preparation behavior and physical properties of PLGA nanoparticles. From all the experimental results obtained, the optimum preparative conditions of PLGA nanoparticles and the role of PVA on the formation of polymeric nanoparticles were clarified by considering PVA-PLGA interaction.

2. Materials and methods

2.1. Materials used

PLGA with average molecular weight of 47 000, whose copolymer ratio of DL-lactide to glycolide is 85:15 (Medisorb®, Du Pont, USA), was used as

received. Twelve grades of PVA (Poval®, Kuraray Co., Japan) with different degrees of hydrolyzation and polymerization (shown in Table 1) were used as received. All other chemicals and solvents were of reagent grade.

2.2. Preparation of aqueous PLGA nanoparticle dispersions

Aqueous PLGA nanoparticle dispersions were prepared according to the SESD method developed by Niwa et al. (1993). Two hundred mg of PLGA were dissolved in the mixture of 25 ml of acetone and 0.5 ml of dichloromethane. The resultant polymer solution was added to 50 ml of aqueous PVA solution (4%, w/w) using a peristaltic pump at 2.0 ml/min while stirring continuously at 400 rpm. The organic solvents were then evaporated in a vacuum for 3–4 h to solidify the emulsion droplets. The nanoparticles formed were separated by ultracentrifugation (156 200 × g 1 h; CP-56G, Hitachi Koki, Japan). The nanoparticles obtained were redispersed into a small volume of

Table 1
Various commercial grades of PVA[a] used in this study

PVA grade	Hydrolyzation degree[b] (%)	Polymerization degree[c]
PVA		
105	98.5	500
110	98.5	1000
117	98.5	1700
120	98.5	2000
PVA		
205	88.0	500
210	88.0	1000
215	88.0	1500
217	88.0	1700
PVA		
403	80.0	300
405	80.0	500
417	80.0	1700
420	80.0	2000

[a] Chemical structure: $-(CH_2CHOH)_l-(CH_2CH(OCOCH_3))_m$
[b] Hydrolyzation degree $= l/(l+m)$.
[c] Polymerization degree $= l+m$.

purified water while stirring. The reconstituted aqueous dispersion was passed through a 1.0-μm membrane filter (FR-100, Fuji Photo Film, Japan) to remove aggregates. The yield of PLGA nanoparticles was represented by the weight fraction % of the nanoparticles passed through a membrane filter (1.0 μm) to the total weight of PLGA fed into the system.

2.3. Particle size measurement

The mean diameter of the PLGA nanoparticles was measured by means of a laser particle analyzer (LAP 3100, Otsuka Electronics, Japan) with a photon correlator (LPA 300, Otsuka Electronics, Japan).

2.4. Minimum film-forming temperature (MFT) measurement

MFT of aqueous PLGA nanoparticle dispersion was determined with a micro-melting point apparatus (Yanagimoto Seisakusho, Japan). One droplet of the aqueous dispersion containing 2% w/w PLGA nanoparticles was placed on a small glass plate using a Pasteur pipette and then heated at 2°C/min. The temperature, at which a droplet began to become a transparent film, was recorded as the MFT (Keshikawa and Nakagami, 1994).

2.5. Redispersibility test of freeze-dried nanoparticles into aqueous phase

Four ml of aqueous PLGA nanoparticle dispersions were filled into a vial for injection and then freeze-dried in a vacuum. The dried PLGA nanoparticles obtained were redispersed by adding 4 ml of purified water into the vial. The redispersibility was evaluated in terms of the ratio of mean diameter of the reconstituted nanoparticles to that of the original nanoparticles.

2.6. PVA gelatinization study

Various commercial grades of PVA with different degrees of hydrolyzation and polymerization were used for this study. A predetermined volume of acetone was added into a vessel containing a
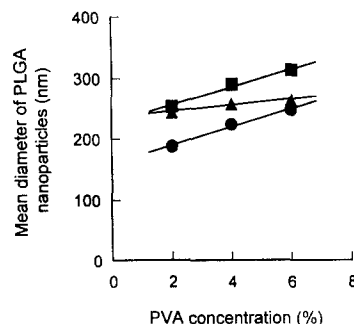

Fig. 1. Mean diameter of PLGA nanoparticles as a function of PVA concentration. The degrees of hydrolyzation of PVA were (●) 98.5%, (▲) 88.0%, and (■) 80.0%, and the degrees of polymerization were 1700.

predetermined volume of aqueous PVA solution and the mixture was gently shaken by hand. After the system stood for a while, the formation of the PVA gel phase was optically checked. The PVA gel formed was carefully taken out from the vessel and was weighed after drying in an oven at 80°C for 12 h. In this study, the volume of organic solvent and the grade of PVA were appropriately altered according to the experimental design.

3. Results

3.1. Effect of PVA grade on the formation of nanoparticles

Several batches of PLGA nanoparticles were prepared using three commercial grades of PVA (PVA117, PVA217 or PVA417), each of which has the same degree of polymerization but has the different degree of hydrolyzation as shown in Table 1. In spite of the identical operating procedure applied, the size of the PLGA nanoparticles obtained varied depending on the PVA grades. In Fig. 1, the changes in the mean diameter of PLGA nanoparticles are shown as a function of PVA concentration in the aqueous phase. As a general tendency, their diameters increased when the concentration of PVA was increased. This was clearly observed in the PVA117 and PVA417 grades, whereas the concentration dependencies with PVA217 were small. Also, it was a notable
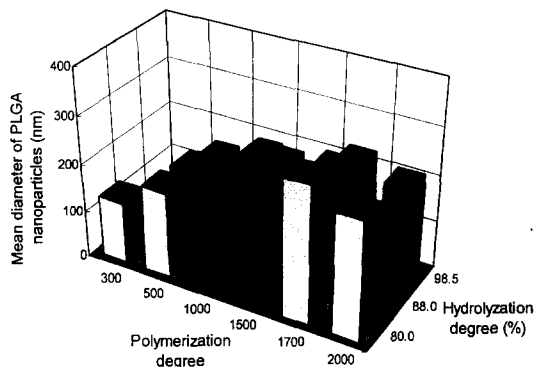
Fig. 2. Three-dimensional plots of mean particle size of PLGA nanoparticies against the degrees of hydrolyzation and polymerization of PVAs.
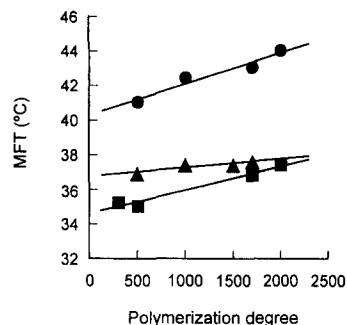

Fig. 3. Effect of polymerization degree of PVA on minimum film-forming temperature of aqueous PLGA nanoparticle dispersions. The degrees of hydrolyzation of PVA were (●) 98.5%, (▲) 88.0%, and (■) 80.0%.

finding that the mean particle size became smaller with the increasing of the degree of hydrolyzation of PVA at the concentration of 2 to 6%.

The effect of the degree of polymerization on particle size was investigated using various commercial grades of PVA. The three-dimensional plots of mean particle size of PLGA nanoparticles against the degrees of hydrolyzation and polymerization of PVAs are shown in Fig. 2. The mean diameter of the PLGA nanoparticles increased with the degree of polymerization of PVA at any degree of hydrolyzation, and trended to slightly decrease at 2000 of polymerization degree. The size distributions of PLGA nanoparticles, which were prepared with various concentration of PVA solution (Fig. 1) and various commercial grades of PVA (Fig. 2), were monomodal.

The yields of PLGA nanoparticles, when various commercial grades of PVA were employed, are listed in Table 2, where the yield % represents the preparation efficacy of PLGA nanoparticles, defined as the weight % of submicron-sized particles to the total PLGA fed into the system. The data clearly indicated that the use of highly hydrolyzed grade of PVA resulted in the low yield of nanoparticles, and the visual inspection proved that such low yield was mainly caused by the coagulation among the PLGA nanoparticles during the preparation process. The degree of polymerization had essentially no effect on the preparation efficacy.

3.2. Effect of the PVA grade on the properties of PLGA nanoparticles

To clarify the effects of the PVA grade on the physical properties of PLGA nanoparticles, the MFT of each preparation was determined. In Fig. 3, the MFTs are plotted against the degree of polymerization of the PVAs with different degree of hydrolyzation. The MFT increased with the increasing of the degree of polymerization of the PVA. It was also found that the MFT became higher with the increasing of the degree of the hydrolyzation of the PVA.

PLGA is known gradually degraded in the aqueous phase by hydrolysis even under a mild storage condition (Lewis, 1990), so that powderization, e.g. freeze-drying of PLGA nanoparticles, should be necessary to assure the chemical stability when nanoparticles are used as drug carriers or

Table 2
Yield % of PLGA nanoparticles prepared using various PVAs

Polymerization degree	Hydrolyzation degree		
	98.5%	88.0%	80.0%
300			103.4
500	38.5	106.7	103.6
1000	36.7	102.3	
1500		99.2	
1700	44.0	89.2	102.2
2000	40.3		106.3

pharmaceutical excipients. The redispersibility of the freeze-dried PLGA nanoparticles, prepared using various commercial grades of PVA, was evaluated as shown in Fig. 4, exhibiting the three-dimensional plots of redispersibility against the degrees of hydrolyzation and polymerization. Since the redispersibility was defined as the ratio of the mean diameter of the rehydrated nanoparticles to that of original nanoparticles, the small value means good redispersibility. When PVA with lower degree of hydrolyzation was used, an excellent redispersibility was found.

4. Discussion

It was found that the formability and physical properties of PLGA nanoparticles depended mainly on the type of PVA formulated. This finding strongly suggests that some amount of PVA might be adsorbed on the surface of PLGA nanoparticles during preparation, determining their surface characteristics. This speculation was strongly supported by a linear change in mean particle size of nanoparticles by increasing PVA concentration, as shown in Fig. 1. The physical properties, e.g. mobility or gelatinization, of the adsorbed PVA should determine the preparation behavior of nanoparticles and their micromeritic properties.
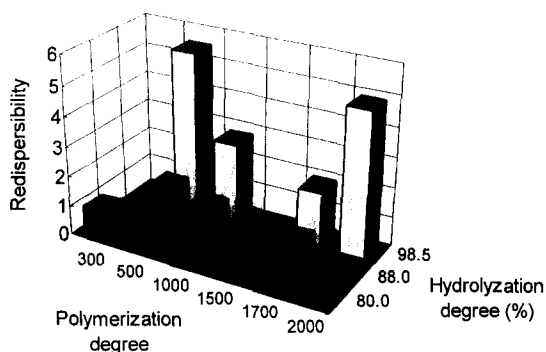
Fig. 4. Three-dimensional plots of redispersibility of freeze-dried nanoparticles after rehydration against the degrees of hydrolyzation and polymerization of PVAs.
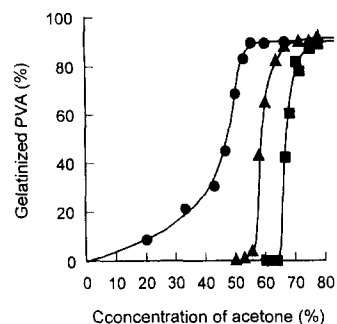
Fig. 5. PVA gelatinization as a function of concentration of acetone. The degrees of hydrolyzation of PVA were (●) 98.5% (▲) 88.0%, and (■) 80.0%, and the degrees of polymerization were 1700. The concentration of PVA solution was 4% (w/w).

4.1. Gelatinization of PVA

It has been often pointed out that the viscosity of aqueous PVA solution varies with the introduction of water-miscible organic solvent, resulting in unique viscoelastic behavior (Nagano et al., 1970). This phenomenon indicates that, in the SESD method in this study, the water-miscible organic solvent (i.e. acetone) diffused from emulsion droplets might change the physical properties of PVA molecules adsorbed at the oil-water interface of emulsion droplets. Thus the gelatinization study of PVA, in which acetone was introduced into the aqueous PVA solution to detect the gel phase deposition, was performed. In Fig. 5, the precipitated % of PVA gels is plotted against the amount of acetone added into the aqueous solutions for three commercial grades of PVA (PVA117, PVA217 and PVA417). The amount of precipitated gel was found to increase sigmoidally up to around 100% by increasing the concentration of acetone in each PVA grade. It was a notable finding that highly hydrolyzed PVA was easily gelatinized even at lower concentration of acetone.

The concentration of acetone, at which 50% of PVA was gelatinized (precipitated), was defined as $C_{50\%}$, and the amount of precipitated PVA gel was determined at $C_{50\%}$ to examine the effect of the degree of polymerization on the gelatinization. Fig. 6 exhibits the amount of precipitated PVA gel as a function of the degree of polymerization.

With an increase of the degree of polymerization, the amount of precipitated gel increased at each degree of hydrolyzation.

In the SESD method, the acetone used as a water-miscible solvent in the oil phase diffused constantly toward the aqueous phase through the oil-water interface. Therefore, the gelatinization behavior of PVA by contacting acetone on the surface of emulsion droplets should be a key factor to control the preparation behaviors of PLGA nanoparticles and their properties. Further, poorer formability of nanoparticles with highly hydrolyzed PVA shown in Table 2 suggested that the inter- and intra-interaction of PVA molecules deposited on the emulsion droplets should be another important factor.

4.2. Role of PVA deposited on PLGA nanoparticles

All phenomena observed in this study might be rationally explained by the PLGA- and PVA-PVA interaction on the surface of emulsion droplets. The schematic representations of the structure of PVA-PLGA nanoparticle in the aqueous phase are proposed in Fig. 7. Hydrocarbon chains of PVA are possibly adsorbed on the surface of nanoparticles via hydrophobic bonding, and a large number of hydroxyl groups of PVA could be hydrated at the surface to stabilize nanoparticles. This could be explained by the fact that preparation behaviors of nanoparticles were determined more effectively by the degree of hy-

Fig. 7. Schematic representation of surface structure of PLGA nanoparticle prepared using PVA with (A) high and (B) low degree of hydrolyzation.

drolyzation than by the degree of polymerization. Owing to strong hydrogen bonds via hydroxyl groups between inter- or intra-molecules of PVA, the hydrophylicity of PVA in the aqueous phase decreases with the increasing of the degree of hydrolyzation (Nagano et al., 1970). Acetone, which diffused from the oil phase in the nanoparticle preparing process, may hinder the hydration of PVA at the interface, resulting in the creation of a polymer-polymer, i.e. PVA-PVA, network. When the degree of hydrolyzation of PVA is higher, the hydration of hydroxyl groups would be further hindered due to increasing the hydrogen bonds between inter- and intra-molecules (Fig. 7A). This may produce aggregated nanoparticles, leading to a decrease in the yield of nanoparticles as shown in Table 2. Furthermore, strong aggregation during freeze-drying made it difficult to rehydrate the dried nanoparticles in the aqueous phase, resulting in poor redispersibility as shown in Fig. 4.

On the other hand, when low-hydrolyzed PVA was applied, the hydrogen bonding between the hydroxyl groups in the PVA molecule adsorbed PLGA nanoparticles could be reduced by the steric hindrance of acetoxyl groups. Therefore, they are more hydrophylized in the aqueous phase, producing stable dispersions. Accordingly, to induce the gelatinization of low-hydrolyzed PVA by reducing the hydrophylization in the aqueous phase, a higher concentration of acetone was required to be introduced into the system as
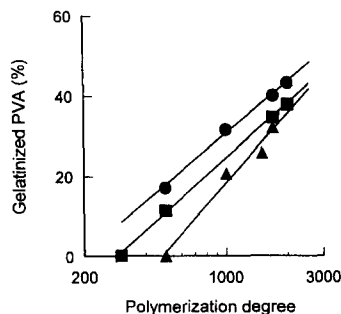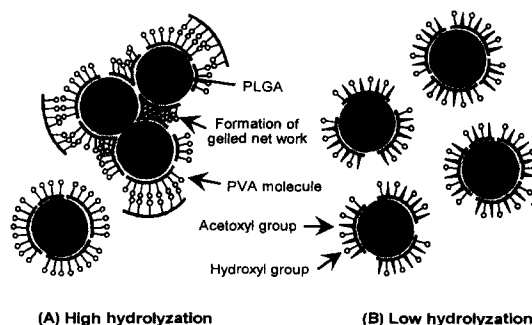

Fig. 6. PVA gelatinization as a function of degree of polymerization. The degrees of hydrolyzation of PVA were (●) 98.5% (▲) 88.0%, and (■) 80.0%. The concentration of PVA solution was 4% (w/w).

shown in Fig. 5. Around the PLGA nanoparticles prepared using low-hydrolyzed PVA, a hydrated layer was formed as depicted in Fig. 7B, resulting in a higher yield of nanoparticles (Table 2) and better redispersibility (Fig. 4). The hydrated layer on the surface of the PLGA nanoparticles lowered MFT as shown in Fig. 3, because water works as a plasticizer in polymeric film-forming system (Lehmann, 1989).

5. Conclusions

It was clarified that the PLGA nanoparticles preparation and their physical properties were determined by the type of PVA used as an emulsion stabilizer. The use of low-hydrolyzed PVA was useful to increase the productivity of PLGA nanoparticles, e.g. in a high yield and to make uniform their size distribution. In addition, excellent redispersibility of freeze-dried nanoparticles was provided. On the other hand, the use of highly hydrolyzed PVA resulted in unsatisfactory productivity and physical properties of nanoparticles. The results of the gelatinization study of PVA suggested that PVA could be gelatinized preferentially at the oil-water interface in the nanoparticle forming process. The gelatinization could occur significantly when highly hydrolyzed PVA was applied and this would cause the poorer formability of nanoparticles as well as undesirable physical properties, e.g. poor redispersibility. Also, it was found that the influence of the degree of hydrolyzation was more significant than that of the degree of polymerization on the preparation behaviors of nanoparticles. In conclusion, proper selection of the PVA grade was a key point to perform successful particle design of PLGA nanoparticles.

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