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Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method

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Abstract

Purpose: The objectives of this study were to establish a new preparation method for poly(DL-lactide-co-glycolide) (PLGA) nanoparticles by modifying the spontaneous emulsification solvent diffusion (SESD) method and to elucidate the mechanism of nanoparticle formation on the basis of the phase separation principle of PLGA and poly(vinyl alcohol) (PVA) in the preparation system. *Methods:* PLGA nanoparticles were prepared by the modified-SESD method using various solvent systems consisting of two water-miscible organic solvents, in which one solvent has more affinity to PLGA than to PVA and the other has more affinity to PVA than to PLGA. The yield, particle size, size distribution and PVA content of the PLGA nanoparticles were evaluated, and the phase separation behaviors of the polymers were elucidated. *Results:* The modified-SESD method provided a good yield of PLGA nanoparticles over a wide range of composition ratios in the binary mixture of organic solvents. Several process parameters, including the fed amount of PLGA, PLGA concentration and PVA concentration were examined to achieve the optimum preparation conditions. The discrete powder of PLGA nanoparticles was obtained by freeze-drying. No change in the PVA content of PLGA nanoparticles was observed even after several times of washing treatment by ultrafiltration, suggesting a strong surface adsorption. It was found that the appropriate selections of binary solvent mixtures and polymeric concentrations in both organic and aqueous phases could provide excellent yield and favorable physical properties of PLGA nanoparticles. *Conclusion:* The proposed modified-SESD method can be used to provide PLGA nanoparticles of satisfactory quality at an acceptable yield for industrial purposes.

Keywords: PLGA; Poly(vinylalcohol); Nanoparticle; Binary solvents; Coacervation

1. Introduction

Because of their desirable biocompatible and biodegradable properties, poly(lactide-co-glycol-

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ide) (PLGA) and poly(lactide) (PLA) have been widely studied for use as microsphere/implant vehicles for long-term sustained-release preparations (Lewis, 1990). Increasing attention has also been paid to the colloidal particles of these polymers as injectable drug carriers which would enable a long systemic circulation. The pharmaceutical application of such nano-sized particles has been extended to the field of non-parenteral deliveries of drugs via pulmonary, nasal or oral routes. Besides such particulate drug delivery systems, the possibility of using nano-sized particles as a new excipient for solid dosage forms has been explored, which includes pharmaceutical coating (Bindschaedler et al., 1986; Frisbee and McGinity, 1994; Schade et al., 1995), matrix formers (Cohen and Bodmeier, 1988; Bodmeier and Cohen, 1989), a surface modifier (Kawashima et al., 1998) and binding agents (Omelczuk and McGinity, 1992, 1993, 1995). The results of these studies suggested that the extremely small size and large surface area of polymeric particles make it possible to provide new functions to the dosage forms.

Under such circumstances, various methods have been proposed for the preparation of PLGA and PLA nanoparticles (Fessi et al., 1989; Bodmeier and Cohen, 1990; Allémann et al., 1992). One promising technique is the spontaneous emulsification solvent diffusion (SESD) method (Niwa et al., 1993), in which nano-sized particles of PLGA or PLA can be effectively obtained by pouring the polymeric organic solution into an aqueous phase with moderate mechanical stirring. One technical characteristic of this method is the use of a binary mixture of a water-miscible organic solvent such as acetone and a water-immiscible solvent such as dichloromethane as the solvent of the polymeric solution, and the particles are formed via an emulsification process and a subsequent solvent-evaporation process. However, our recent preliminary study regarding the large-scale production of PLGA nanoparticles suggests that the original SESD method sometimes causes a severe aggregation in the particle formation process when the polymeric concentration is increased to an acceptable range for industrial purposes. This indicates that the SESD method should be modified.

The purpose of our present study was to develop a new preparation method applicable to the pharmaceutical production of PLA or PLGA nanoparticles satisfying pharmaceutically acceptable criteria with respect to residual solvent, processing aids, batch-to-batch reproductivity, scale-up, and yields. We modified the original SESD method to improve the applicability to large-scale production. The major modification points of the process were that: (i) a mixture of two water-miscible organic solvents was used for the solvent of the polymeric solution instead of the mixture of water-miscible and water-immiscible organic solvents; and (ii) exclusively low hydrolyzation and polymerization grade of poly(vinylalcohol) (PVA; Murakami et al., 1997), i.e. Poval®-403, was used in this new method as a quasi-emulsifier in the aqueous phase, which closely correlated to the nanoparticle-formation mechanism. Various process parameters influencing the yield of nanoparticles were examined in comparison with the original SESD method. Furthermore, the formation mechanism of nanoparticles is also discussed on the basis of phase separation phenomena of PLGA and PVA.

2. Materials and methods

2.1. Materials

PLGA with an average molecular weight of 46 770, whose copolymer ratio of DL-lactide to glycolide is 85:15 (Medisorb®, Du Pont Co., USA), was used as received. PVA with an 80%-hydrolyzation degree and a 300-polymerization degree (Poval®-403, Kuraray, Japan) was used as a quasi-emulsifier. All other chemicals and solvents were of reagent grade.

2.2. Preparation of PLGA nanoparticles

Fig. 1 is the comparison of the preparation procedure of PLGA nanoparticles between the SESD method (Niwa et al., 1993) and the new method (modified-SESD method). In the original SESD method, PLGA is dissolved in the organic solvent mixture consisting of dichloromethane

and acetone, which are a less water-miscible organic solvent and a freely water-miscible organic solvent, respectively. The polymeric solution obtained above is slowly poured into the emulsifier-containing aqueous phase with agitating by a stirrer. Nanoparticles are formed via the following steps: when the polymeric solution is added, emulsion droplets are formed in the aqueous phase; acetone quickly diffuses out from each emulsion droplet, drastically reducing its size to nano-order; and the consequent 'solvent-evaporation' process, in which the remaining dichloromethane is removed from the system, makes the droplets solidify to finally form polymeric nanoparticles.

This process seems rational, based on physicochemically interesting phenomena. However, due to a considerable amount of residual dichloromethane, the particles are likely to aggregate during the solvent-evaporation process. Therefore, when a larger amount of polymeric solution is used, the aggregation cannot be prevented any more because the probability of collision between particles in the aqueous phase would increase. In addition, the recovering and purifying process in the original SESD method would have to be improved, since the ultracentrifugation process ($156\,200 \times g$, 1 h) was unacceptable for an industrial process in large-scale production. Further, it is necessary to avoid the use of a chlorinated solvent such as dichloromethane because of its toxicity.

Our recent study proved that the use of PVA with low hydrolyzation and polymerization prevented the local gelatinization of PVA at the surfaces of emulsion droplets induced by the diffusion of acetone, which effectively restricted the aggregation of nanoparticles (Murakami et al., 1997). It was considered that the particular commercial grade of PVA, i.e. Poval®-403, was the most suitable for the preparation of nanoparticles. Recently, it was further found that the addition of alcohol to acetone in PLGA organic solution could restrict the local gelatinization of PVA more effectively.

In the modified-SESD process, the mixture of two water-miscible organic solvents, such as

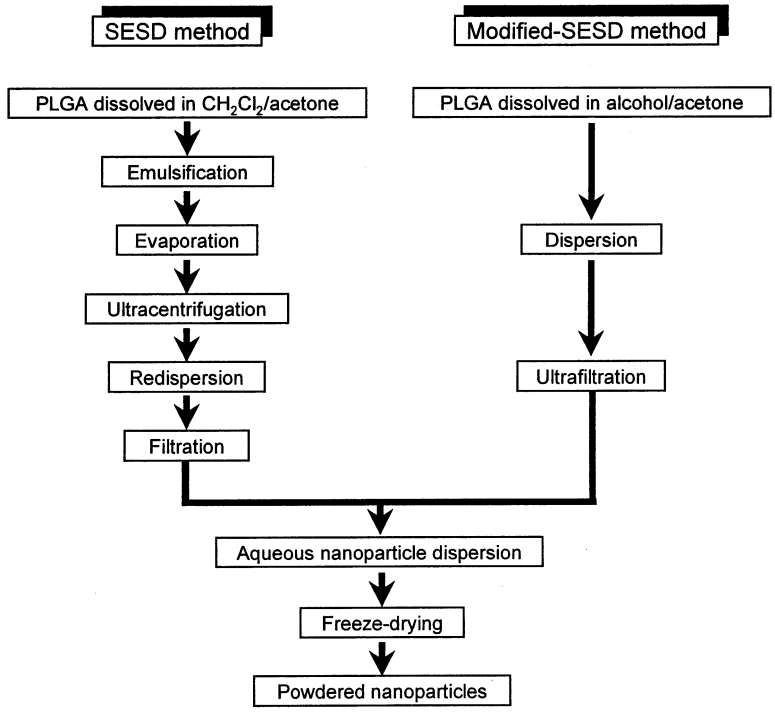

Fig. 1. Preparation procedures of the original SESD method and the modified-SESD method.

ethanol/acetone or methanol/acetone was employed, instead of using the mixture of dichloromethane and acetone. This alteration prevented the aggregation of particles even at a high fed amount of polymeric solution, resulting in improvement in yield as acceptable for industrial purposes. This alteration also provided some additional advantages; for instance, the use of a toxic solvent such as dichloromethane could be avoided in the preparation process; the recovering and purifying process could be simplified by using ultrafiltration technique to omit the solvent-evaporation process; and uniform nanoparticle dispersion could always be attained by even mild agitation.

The typical operating procedure was as follows:

1. Quasi-emulsification: 500 mg of PLGA were dissolved in 12.5 ml of the solvent mixture consisting of acetone plus dichloromethane, ethanol, or methanol. The polymer solution obtained was then added into 50 ml of aqueous PVA solution (4%, w/w) in a 100-ml glass flask using a peristaltic pump at a flow rate of 2.0 ml/min while continuously stirring at 400 rpm with a propeller mixer.
2. Purification: the dispersion formed was transferred into a 1000-ml glass flask and purified water was added up to 500 ml. The dispersion was condensed to 25 ml by means of an ultrafiltration (Minitan® system, Millipore, Japan), and purified water was added up to 500 ml. This dilution–condensation process was repeated three times to remove the residual PVA and organic solvents.
3. Freeze-drying: the aqueous dispersion condensed to 25 ml was passed through a 100-mesh sieve to remove aggregates and then freeze-dried in a vacuum to obtain powdered nanoparticles. The yield of nanoparticles was represented by the percent weight fraction of the powdered nanoparticles relative to the total weight of PLGA used for the preparation.

2.3. Particle size and size distribution measurement

The mean diameter of the PLGA nanoparticles in aqueous dispersion was measured by means of a laser particle analyzer (LAP 3100, Otsuka Electronics, Japan) with a photon correlator (LPA 300, Otsuka Electronics). When the diameter was expected to exceed 1 μm, a laser-based time-of-transition system (Cis-1, Galai Inc., Israel) was used. The mean diameter of freeze-dried nanoparticles was determined after they were dispersed in purified water.

2.4. Measurement of PVA content in PLGA nanoparticles

The PVA amount adsorbed on the surface of PLGA nanoparticles was determined according to E. Allémann's method (Allémann et al., 1993). Two hundred milligrams of freeze-dried nanoparticles was dissolved in 25 ml of chloroform with sonication for 15 min. The organic solution was filtrated using a membrane filter (PTFE filter, ADVANTEC Co., Japan), and the residual PVA on the filter was washed with a small portion of chloroform. After being dried under atmospheric pressure, the filter was transferred into a 200-ml glass flask. One hundred grams of purified water was added to the flask and heated to dissolve the PVA. After removing the filter and cooling, the weight of the PVA solution was adjusted to 100 g. Fifteen milliliters of a 4% (w/w) boric acid solution and 3 ml of an iodine solution (1.27% (w/w) iodine and 2.5% (w/w) potassium iodide in purified water) were added to 5.0 ml of the PVA solution. The volume was adjusted to 50.0 ml with purified water. The solution was filtrated with a 0.5-μm cellulose acetate filter (ADVANTEC). The absorbance of the solution was assayed spectrophotometrically ($\lambda = 620$, 690 nm).

2.5. Cloud point titration for PVA and PLGA

To evaluate the affinity of PLGA to methanol and ethanol, the cloud points were determined applying the titration technique (Haugsberger and DeLuca, 1995). Two milliliters of PLGA acetonic solution at various concentrations was poured into a 20-ml glass test tube. The resultant PLGA solution was slowly titrated with either methanol or ethanol to the cloud point, where the PLGA began to precipitate, as noted by a faint but

distinct cloudiness. The alcohol percentage in the PLGA solution at the cloud point, CL_{alc}, was determined as the index representing the affinity between PLGA and the solvent.

For PVA, 2 ml of aqueous PVA solution (4%, w/w) in the 20-ml glass test tube was titrated with the solvent mixture of methanol/acetone or ethanol/acetone. The volume of solvent mixture required for reaching the cloud point was determined. The volume percentage of solvent mixture at the cloud point, CL_{binary}, was determined as the index representing the affinity between PVA and the solvent mixture.

2.6. Scanning electron microphotography

The morphology of the nanoparticles was observed by means of a scanning electron microscope (JSM-T330A, Nihon Denshi, Japan).

3. Results and discussion

3.1. Effect of composition in binary solvents on yield of nanoparticles

The yield of nanoparticles by the new method was evaluated under various operating conditions. The yields and mean particle size of the nanoparticles are shown as a function of solvent composition in Figs. 2 and 3, compared with those obtained by the original SESD method. The solvent composition was commonly expressed as the acetonic concentration (Φ_{ac}) in both methods, even though the binary solvent system differed.

It was clearly shown that the original SESD method could produce nanoparticles in only a very limited region of solvent composition ($\Phi_{ac} >$ 90%). Although the maximum yield was given at the Φ_{ac} of 98%, the value was a quite dissatisfactory low level, 45%. Such poor yield could be due to the aggregation occurring during the preparation process, as was suggested by the data shown in Fig. 3, in which the mean diameter exceeded 40 μm at the lower Φ_{ac}, and the desirable nano-sized particles were obtained only at higher acetonic concentration ($\Phi_{ac} > 96\%$). With the modified-SESD method, both solvent systems (ethanol/acetone and methanol/acetone) provided nano-sized particles over a wide range of acetonic concentrations where PLGA is completely soluble in the solvent mixture ($\Phi_{ac} > 50\%$). The highest yields (more than 90%) were observed in the range of Φ_{ac} from 50 to 70%. These results indicated that the addition of alcohol to PLGA acetonic solution could prevent the aggregation effectively.

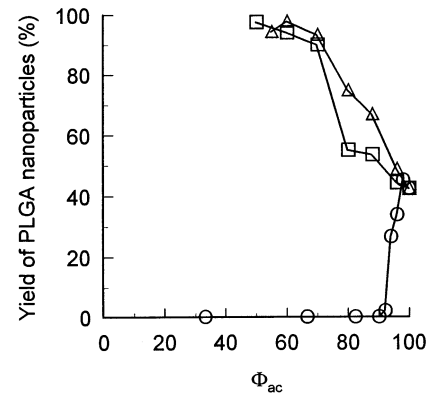

Fig. 2. The yield of PLGA nanoparticles as a function of acetonic concentration. (○) Original SESD method (solvent: dichloromethane/acetone), (△) modified-SESD method (solvent: ethanol/acetone), (□) modified-SESD method (solvent: methanol/acetone).

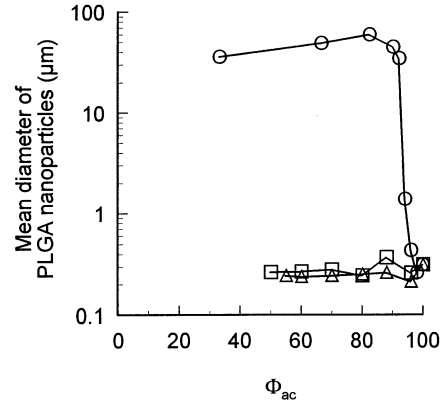

Fig. 3. The mean diameter of PLGA nanoparticles as a function of acetonic concentration. (○) Original SESD method (solvent: dichloromethane/acetone), (△) modified-SESD method (solvent: ethanol/acetone), (□) modified-SESD method (solvent: methanol/acetone).

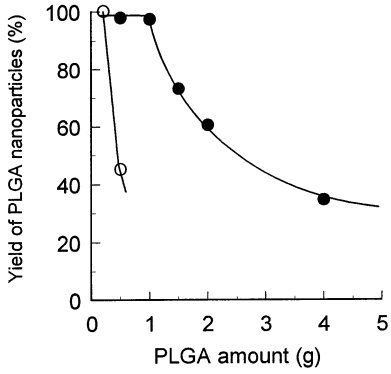

Fig. 4. Effect of the fed amount of PLGA on the yield of nanoparticles. (●) Modified-SESD method (solvent: ethanol/acetone = 4/6), (○) original SESD method (solvent: dichloromethane/acetone = 4/96).

3.2. Variation of process parameters

With this manufacturing technique, the several process parameters, including the fed amount of PLGA, PLGA concentration and PVA concentration, were examined to achieve the optimum preparation conditions in the ethanol/acetone (4/6) system. Fig. 4 shows the change of the yields of nanoparticles with the fed amount of PLGA at constant concentration of the PLGA solution. Even though an excellent yield (almost 100%) was achieved at the fed amount of 0.1 g with the original SESD method, the yield decreased rapidly when the fed amount was raised to 0.5 g. In contrast, the modified-SESD method, with the ethanol/acetone (4/6) system, provided an excellent yield even at fed amounts as high as 1.0 g, and the particle size obtained was around 300 nm in all cases (data not shown). Those findings suggest that the modified-SESD method is more suitable than the original SESD method for the large-scale production of PLGA nanoparticles.

The yields of nanoparticles are shown as the function of PLGA concentration in Fig. 5. Although a satisfactory yield was obtained at PLGA concentrations lower than 4%, the value decreased with the increase in the PLGA concentration. This was probably caused by the increasing viscosity and hence resulting poor dispersibility of the PLGA solution into the aqueous phase.

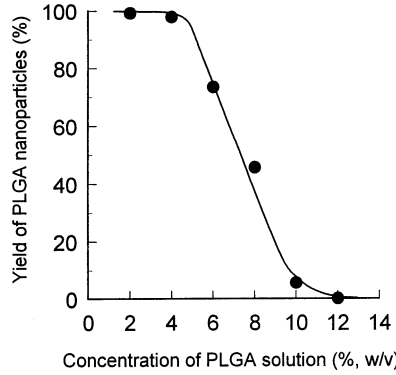

Fig. 5. Effect of PLGA concentration on the yield of nanoparticles prepared in ethanol/acetone (Φ_{ac}: 60%) system.

The effect of the PVA concentration in the aqueous phase on the yield of nanoparticles is shown in Fig. 6. An 8% PLGA solution, even though it was not an optimum condition, was purposely used for this experiment to detect the difference in the yield. Data indicate a maximum yield at PVA concentrations within a range of about 7–15%. At lower concentrations, decreasing cacervation of PVA on the nanoparticle surface could be responsible for lower yields. On the other hand, the precipitation of PLGA around the propeller used in agitation was immediately observed when the PLGA solution was pored into solutions containing more than 15% PVA. This phenomenon could be caused by the worse dispersion of PLGA solution into aqueous phase due to the increase of viscosity of PVA solution.

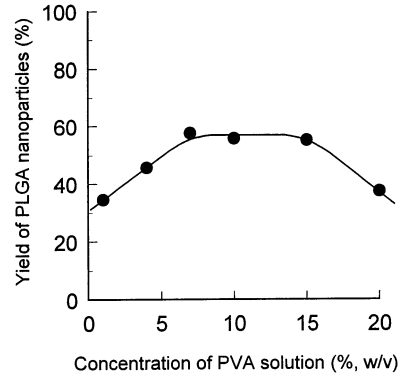

Fig. 6. Effect of PVA concentration on the yield of nanoparticles prepared in ethanol/acetone (Φ_{ac}: 60%) system. PLGA concentration: 8%.

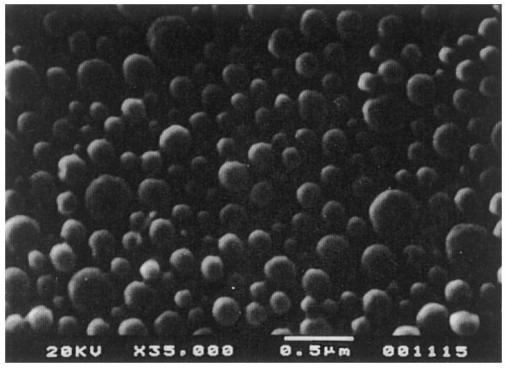

Fig. 7. Typical scanning electron microphotograph of PLGA nanoparticles prepared in ethanol/acetone (Φ_{ac}: 60%) system.

3.3. The characteristics of PLGA nanoparticles prepared by the modified-SESD method

The powder characteristics of the PLGA nanoparticles prepared by the modified-SESD method were evaluated. A scanning electron microphotograph of freeze-dried nanoparticles prepared with the ethanol/acetone (4/6) system is shown in Fig. 7 as a typical example. The PLGA nanoparticles were spherical, discrete particles without aggregation, and smooth in surface morphology, with a diameter of less than 500 nm. The particle sizes of PLGA nanoparticles before and after freeze-drying are compared in Table 1. The small polydispersity index suggested that the size-distribution of the products is fairly monomodal. It was also found that the characteristics of the nanoparticles were not affected by freeze-drying or rehydration. This property was favorable for storage of powdered PLGA nanoparticles to avoid hydrolysis of PLGA in aqueous dispersion.

Table 1
Diameter and polydispersity of PLGA nanoparticles prepared by the modified-SESD method

Solvent composition	Diameter (nm)		Polydispersity index
	Before[a]	After[b]	
Ethanol/acetone(4/6)	261	264	0.057
Methanol/acetone(4/6)	266	263	0.024

[a] Before freeze-drying.
[b] After rehydration of freeze-dried nanoparticles.

Table 2
Mean diameter and residual PVA amount of PLGA nanoparticles after washing treatment by ultrafiltration

Number of ultrafiltration	Mean diameter (nm)	PVA content (%)
2	292	2.73
3	283	2.40
4	280	2.63
5	284	2.66
6	288	2.46

The above-mentioned monodispersed size distribution and excellent redispersibility of nanoparticles indicate that the surface of PLGA nanoparticles is stabilized by some reasons to prevent aggregation. The most likely reason to explain the findings might be the adsorption of PVA to PLGA nanoparticles, as shown in our previous report (Murakami et al., 1997). Relevant evidence supporting this explanation is shown in Table 2, in which the mean size of nanoparticles and the PVA content of PLGA nanoparticles after the purifying process, i.e. the washing treatment by ultrafiltration repeated two to six times, are compared. The results revealed that the mean diameter and the PVA content did not change even when the washing treatment was repeated, indicating that the surface of each particle had adsorbed a PVA layer strongly.

Regarding the surface adsorption of PVA, Iler (1973, 1975) proposed a model to express the physical adsorption of PVA to colloidal silica particles, in which PVA molecules are fixed on the surface via hydrophilic bonding with the silanoyl groups (Si=O) of colloidal silica. On the other hand, the observed strong adsorption of PVA on the surface of PLGA nanoparticles may be expressed by the following model; that is, the hydroxyl groups of PVA molecules are fixed to the acetyl groups of PLGA via hydrophobic bonding, as shown schematically in Fig. 8. The good rehydration of powdered nanoparticles via freeze-drying without the addition of any cryoprotectant can be explained by the hydrophilicity of PVA

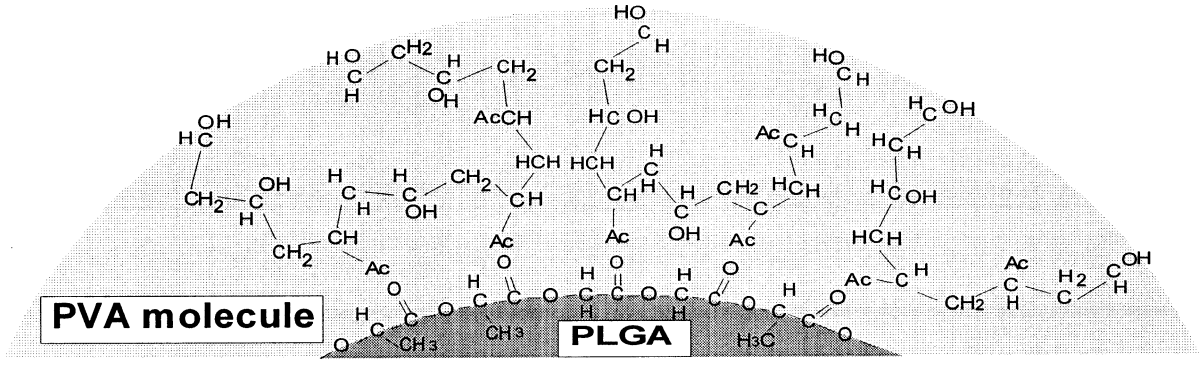

Fig. 8. Schematic representation of molecular orientation of PVA bound to PLGA molecules at the surface of PLGA nanoparticles.

molecules, which were strongly bound to the surface of PLGA nanoparticles (Murakami et al., 1997).

3.4. Phase separation of PLGA and PVA

To elucidate the reasons for the improved yield and superior powder property achieved with the modified-SESD method, the phase separation behaviors of PLGA and PVA were examined. In the ethanol/acetone or methanol/acetone solvent system, alcohol is regarded as the 'poor solvent for PLGA' whereas acetone is regarded as the 'good solvent for PLGA'. Therefore, the cloud point, CL_{alc}, was determined by titrating, until the PLGA solution became cloud.

Fig. 9 shows the phase diagrams as the CL_{alc}–PLGA concentration profiles for the binary solvent systems, ethanol/acetone and methanol/acetone. In both of these binary solvent systems, CL_{alc} was observed to be almost independent of the PLGA concentration, although it was slightly higher at lower PLGA concentrations. However, the CL_{alc} values seemed to be specific to the individual binary solvent system. The alcoholic concentration required for the phase separation from the PLGA acetonic solution was higher in the system with methanol than ethanol. It is also notable that the CL_{alc} values for methanol and ethanol (about 45–55%) were almost coincident with the alcoholic fraction of the binary solvent systems providing the maximum yields in the modified-SESD method (Fig. 2). These results indicate that the phase separation of PLGA plays an important role in the nanoparticle-formation process.

Fig. 10 is the phase diagram of PVA, which was drawn by titrating the alcohol–acetone mixtures with various compositions into the 4% aqueous PVA solution. The CL_{binary} values, the volume percentage of the binary solvents at the cloud point, increased with the increase in the alcoholic fraction of the binary solvent system. The phase separation of PVA occurred intensively when acetone alone was used, but it became milder with the increase in the alcoholic fraction in the binary solvents. Although methanol seemed to have a slightly larger potential than ethanol for preventing the phase separation, the difference in the affinity to PVA between alcohol and acetone must be a more important factor.

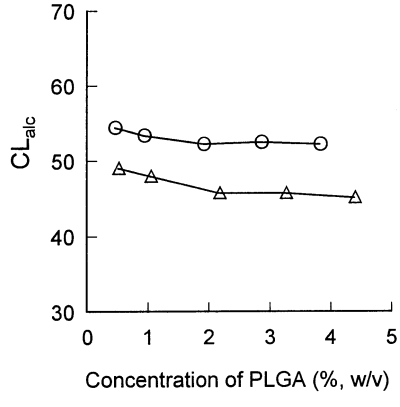

Fig. 9. Changes in cloud point as a function of PLGA concentration. (○) Methanol/acetone, (△) ethanol/acetone.

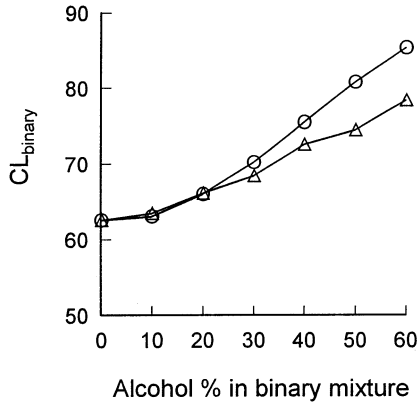

Fig. 10. Changes in cloud point as a function of PVA concentration. (○) Methanol/acetone, (△) ethanol/acetone.

3.5. Mechanism of nanoparticle formation

In the original SESD method, the PLGA nanoparticles are produced via emulsion droplets because of the use of dichloromethane. On the other hand, the technical characteristic of the modified-SESD method consists of the combination of two water-miscible organic solvents; one has higher affinity to PLGA than to PVA (as acetone) and the other has higher affinity to PVA than to PLGA (as alcohol). Therefore, the following different mechanism of the nanoparticle formation is speculated and is schematically proposed in Fig. 11.

The PLGA solution is in a state that the addition of alcohol instead of dichloromethane makes PLGA easy to induce deposition, as is suggested by the data in Fig. 9. When the PLGA solution is dispersed into the aqueous PVA solution (Fig. 11, stage 1), the perturbation of the interface spontaneously produces a larger interfacial area, which leads to nano-sized quasi-emulsion droplets of PLGA solution. This interfacial turbulence would be governed by the well-known Marangoni effect (Sternling and Scriven, 1959). Thus, the alcohol preferentially diffuses out of the droplets since the affinity of alcohol to PLGA is lower than that of acetone (stage 2). Continuously, acetone diffuses out of the droplets and the coacervation of PVA is induced by the increasing concentration of acetone (stage 3). In addition, the PLGA concentration inside increases to induce the PLGA deposition (stage 4). Finally, the subsequent solidification of PLGA and PVA adsorption completes simultaneously (stage 5). As these deposition processes are conducted instantaneously and spontaneously, the uniform nanoparticle dispersion can always be attained even by mild agitation.

4. Conclusions

The present study demonstrated that the original SESD method can be modified to improve the

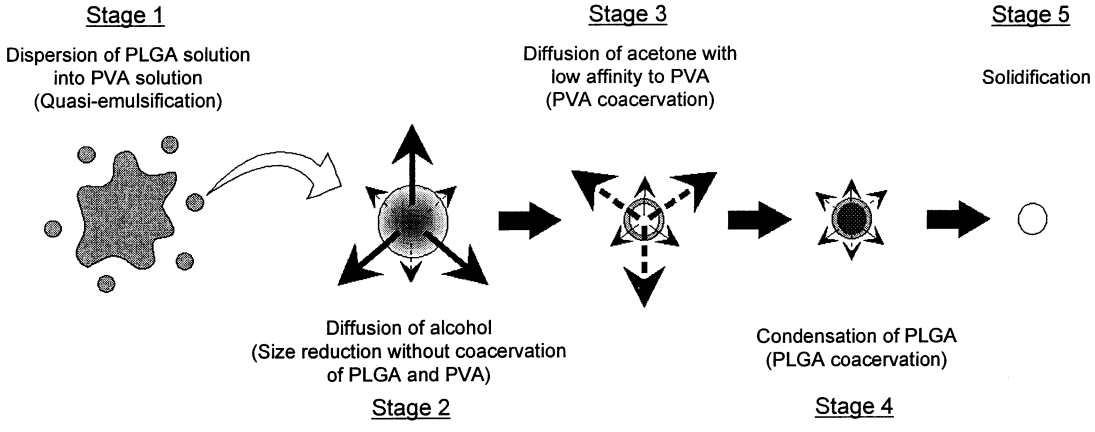

Fig. 11. Possible mechanism of nanoparticle-formation by the modified-SESD method. Solid lines: the diffusion of methanol or ethanol; dotted lines: the diffusion of acetone; bold lines: rapid diffusion; and narrow lines: slow diffusion.

yield by altering the solvent of the polymeric solution from dichloromethane/acetone to the binary mixture of water-miscible organic solvents, i.e. methanol/acetone or ethanol/acetone. The phase diagram study suggested that two sequential phase separations of PLGA and PVA are involved in the nanoparticle-formation process of the modified-SESD method, which may contribute to the considerable improvement of yield. The modified-SESD method can provide a practically acceptable quality of PLGA nanoparticles with high yields by a simple preparation process.

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