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The effect of type of organic phase solvents on the particle size of poly(D,L-lactide-co-glycolide) nanoparticles

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Abstract

Poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles were prepared by an emulsification-diffusion method. To investigate the effect of type of organic phase solvents on the mean particle sizes of obtained PLGA nanoparticles, different organic solvents [ethyl acetate (EA), propylene carbonate (PC), acetone (ACE), and dichloromethane (DCM)] were used with several stabilizers [didodecyl dimethyl ammonium bromide (DMAB), poly(vinyl alcohol) (PVA), and Pluronic F68]. The particle size of nanoparticles was observed by the dynamic light scattering method and atomic force microscopy (AFM). When DMAB, an ionic stabilizer, was used, small PLGA nanoparticles below 70 nm were obtained for EA and PC as partially water-soluble organic solvents, while large PLGA nanoparticles above 290 nm were prepared for ACE and DCM as a fully water-soluble solvent and a water-immiscible solvent, respectively. However, when PVA or Pluronic F68, non-ionic stabilizers, were used, a big difference in mean particle sizes between partially water-soluble solvent or fully water-soluble solvent and water-immiscible solvent was not observed, and all particles showed a large mean diameter above 110 nm, irrespective of the type of organic phase solvents.

Keywords: Nanoparticles; PLGA; Emulsification-diffusion; Organic phase solvents; Stabilizers

1. Introduction

Biodegradable nanoparticles prepared from poly(lactide-co-glycolide) polymers (PLGA) have obtained considerable interest in recent years for their use as a delivery vehicle for various pharmaceutical agents. PLGA is by far the most common biodegradable polymer used for the controlled delivery of drugs due to its early use and approval as compatible biomaterial in humans [1]. Many methods are currently available for the preparation of polymeric nanoparticles, such as the emulsification-evaporation method [2], salting-out procedure [3], and nanoprecipitation method [4]. Nevertheless, several difficulties have been encountered when adopting these methods, such as working with toxic solvents (emulsification-evaporation), non-accepted stabilizers and salts that are incompatible with bioactive compounds (salting-out), and problems with low yields and poor entrapment efficacy (nanoprecipitation) [5]. Also, these techniques were not useful to reduce the particle size and were impossible to produce nanoparticles with diameter less than 100 nm [6].

The emulsification-diffusion method has been used successfully to prepare nanoparticles from preformed polymers in an efficient and reproducible manner [5]. In this method, it is possible to make nanoparticles with diameter less than 100 nm by an optimal combination of organic phase solvents and stabilizers. It involves the formation of a conventional oil-in-water emulsion within a partially water-soluble solvent. The subsequent addition of water to the system makes the organic solvent diffuse into the external phase, resulting in the formation of nanoparticles [7]. Several workers have studied the effects of several preparative variables on particle size, such as the type and concentration of the stabilizers, the stirring speed, and the polymer concentration in the organic phase [6,8,9].

Quintanar-Guerrero et al. [8] prepared poly(D,L-lactic acid) (PLA) nanoparticles by the emulsification-diffusion method.

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They found that an increase in both stirring speed and concentration of stabilizer reduces the size of nanoparticles. Kwon et al. [6] also synthesized PLGA nanoparticles by the emulsification-diffusion method. They reported that as the PLGA concentration in the organic phase increases, the mean size of resultant PLGA nanoparticles increases. Choi et al. [9] thermodynamically studied the emulsification-diffusion method for making PLGA nanoparticles, quantitatively considering the diffusion coefficients (D_{sw}, D_{ws}), exchange ratio ($R = D_{sw}/D_{ws}$), and polymer–solvent interaction parameter (χ). They found that R is directly proportional to the size of the PLGA nanoparticles, while χ is inversely proportional to it.

In these studies, only the partially water-soluble organic solvents, such as ethyl acetate (EA), methyl ethyl ketone (MEK), propylene carbonate (PC), and benzyl alcohol (BA), were used for the preparation of PLGA nanoparticles. However, the influence of other organic solvents, such as acetone (ACE) as a fully water-soluble solvent or dichloromethane (DCM) as a water-immiscible solvent, has not been considered in the formation of PLGA nanoparticles. This study describes the effect of various organic solvents including partially water-soluble solvent, water-immiscible solvent, and fully water-soluble solvent on the particle size of PLGA nanoparticles prepared by the emulsification-diffusion method.

2. Experimental

2.1. Materials

Biodegradable PLGA (75:25 lactide:glycolide, MW 75,000–120,000) was purchased from Sigma Chemicals. The organic solvents, dichloromethane (DCM), ethyl acetate (EA), propylene carbonate (PC), and acetone (ACE) were HPLC grade purchased from Aldrich Chemicals. The stabilizers, didodecyl dimethyl ammonium bromide (DMAB) and poly(vinyl alcohol) (PVA, MW 9000–10,000, 80% hydrolyzed), were also purchased from Aldrich Chemicals. The stabilizer, Pluronic F68, was purchased from Sigma Chemicals.

2.2. Preparation of nanoparticles

The PLGA nanoparticles were prepared using the emulsification-diffusion method. 100 mg of PLGA was dissolved in 10 ml of various organic solvents (EA, PC, ACE, and DCM). The organic phases were added into 20 ml of an aqueous phase containing stabilizer. After mutual saturation of organic and aqueous phases, the mixture was emulsified for 1 min with a probe-tip sonicator (probe-tip diameter: 1.3 cm, Sonics & Materials Inc., Danbury, CT, USA) operating at 40% amplitude intensity. In order to allow for diffusion of the organic solvent into water, a constant volume (80 ml) of water was subsequently added to the o/w emulsion under moderate magnetic stirring, leading to the formation of PLGA nanoparticles.

2.3. Nanoparticle characterization

The mean particle size and particle size distribution of the nanoparticles were assessed by photon correlation spectroscopy (NICOMP, model 380, CA, USA). Particle size was expressed as volume-weighted mean diameter in nanometers, and was obtained from the measurement of at least three batches of nanoparticles. AFM was used to image the shape and size of the nanoparticles prepared by the emulsification-diffusion method. A drop of diluted aqueous dispersion was placed on a washed microscope slide and dried under vacuum for 24 h. The measurements were performed using a commercial AFM (XE-100, PSIA, Republic of Korea) at room temperature in non-contact mode (frequency = 5 kHz).

3. Results and discussion

In the formation of PLGA nanoparticles by the emulsification-diffusion technique, both the organic solvent phase containing PLGA and aqueous phase containing stabilizer are in the state of thermodynamic equilibrium. The addition of water to the system destabilizes the equilibrium. It causes the organic solvent to diffuse to the external phase. During this transport of the solvent, PLGA nanoparticles are produced, and their size may be dependent upon the type of organic phase solvents. We investigated the effect of the type of organic phase solvents on the mean particle size of PLGA nanoparticles. To evaluate the effect of organic phase solvents, various organic solvents were used for the preparation of PLGA nanoparticles. Table 1 shows a summary of the solubility of the organic phase solvents used for nanoparticle preparation in water [10] or that of the PLGA in organic phase solvents. EA and PC are partially water-soluble and are good solvents for PLGA. ACE is completely miscible with water in all proportions and is a good solvent for PLGA. DCM is immiscible with water and is an excellent solvent for PLGA.

Fig. 1 shows the particle size distributions of the PLGA nanoparticles prepared with different organic phase solvents, when DMAB as a stabilizer was used at a constant concentration of 1% (w/v) with respect to the amount of water. Small particles below 70 nm in mean particle size were obtained using partially water-soluble solvents (EA, PC), while large particles above 290 nm in mean particle size were obtained using

Table 1
Solubility of organic phase solvents in water or PLGA in organic phase solvents

	Ethyl acetate (EA)	Propylene carbonate (PC)	Acetone (ACE)	Dichloromethane (DCM)
Water	Slightly soluble (10% (v/v) at 25 °C)	Slightly soluble (17.5% (v/v) at 25 °C)	Very soluble (infinitely at 25 °C)	Immiscible (2% (v/v) at 25 °C)
PLGA	Good solvent	Good solvent	Good solvent	Excellent solvent

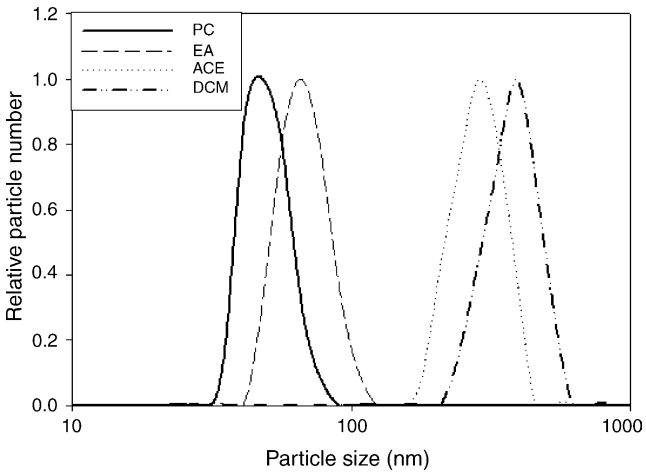

Fig. 1. The effect of different organic phase solvents on the particle size distributions of the PLGA nanoparticles, when DMAB was used as a stabilizer.

fully water-soluble solvent (ACE) and water-immiscible solvent (DCM). Also, the solutions of PLGA nanoparticles obtained from EA and PC as organic phase solvents were more transparent in sight than those of ACE and DCM. This means that the type of organic phase solvents plays an important role in the mean size of nanoparticles when using DMAB as the stabilizer. Since nanoparticles are formed from the emulsion droplets after organic solvent diffusion, their size is dependent on the stability of the emulsion droplets, which collide and coalesce among themselves. When the stabilizer remains at the liquid–liquid interface during the diffusion process, and its protective effect is adequate, nanoparticles will form [8]. Small particle sizes for EA and PC were attributed to both the adequacy of the stabilizer's protection against coalescence, and the low interfacial tension between aqueous and organic phases, resulting from their partially water-soluble nature. However, when DCM was used as the organic phase solvent, nearly every formulation resulted in significant aggregation due to its immiscible nature with water, and the stabilizer DMAB was not able to completely prevent aggregation of emulsion droplets, leading to large mean particle size (390 nm). On the other hand, as ACE is completely miscible with water, stable emulsions between organic and aqueous phases are not formed despite the presence of a stabilizer. Upon mixing the two phases, the PLGA immediately precipitates as sub-micron particles, resulting in large particle size [11].

Fig. 2 shows the AFM images of the PLGA nanoparticles prepared with EA (a), DCM (b), and ACE (c) as organic phase solvents, respectively, when DMAB at 1% (w/v) was used as a stabilizer. The PLGA nanoparticles have spherical shapes with different sizes depending on the type of organic phase solvents. When EA, a partially water-soluble organic solvent, was used, small and discrete particles of 40–70 nm in size were obtained. On the other hand, large and aggregated particles of 350–450 nm in size were observed in DCM as a water-immiscible organic solvent. When ACE, a completely water-soluble organic solvent, was used, large and discrete particles of 250–400 nm in size were obtained. From these AFM images, we can see that smaller nanoparticles below 70 nm are obtained using a partially water-soluble solvent (EA) than when using a water-immiscible solvent (DCM) or a fully water-soluble solvent (ACE). This is in agreement with the result of particle size distributions in Fig. 1.

Figs. 3 and 4 show the particle size distributions of the PLGA nanoparticles prepared with different organic phase solvents (EA, PC, ACE, and DCM), when PVA and Pluronic F68 were used as stabilizers at a constant concentration of 1% (w/v), respectively. Contrary to the result of DMAB in Fig. 1, small particle size below 70 nm in mean particle size was not obtained for PC and EA, and no major difference in particle size distributions between partially water-soluble solvent (EA and PC) or fully water-soluble solvent (ACE) and water-immiscible solvent (DCM) was observed in either PVA or Pluronic F68. In particular, the mean particle sizes of PLGA nanoparticles stabilized with PVA showed relatively similar values in Fig. 3, irrespective of the difference in the type of organic phase solvents. It is also remarkable for Pluronic F68 in Fig. 4 that EA shows smaller mean particle size than PC, which showed a different trend with the results of DMAB and PVA in Figs. 1 and 3, respectively. In non-ionic stabilizer systems (PVA and Pluronic F68), particles are stabilized by steric hindrance, but in an ionic stabilizer system (DMAB), particles are stabilized by electrostatic repulsion [6]. The above results mean that the effect of type of organic phase solvents in obtaining small PLGA nanoparticles below 70 nm becomes more significant when particles are stabilized by electrostatic repulsion than steric hindrance.

Table 2 shows the effect of different stabilizers and organic phase solvents on the mean particle sizes of PLGA nanoparticles. The smallest particle (50 nm) was obtained using DMAB (stabilizer) and PC (organic phase solvent), while the largest particle (461 nm) was obtained using Pluronic F68 (stabilizer) and DCM (organic phase solvent). These results indicate that proper choice of organic phase solvent and stabilizer is a key factor in determining the mean diameter of PLGA nanoparticles.

Fig. 5 shows the effect of the type and concentration of stabilizers on the mean size of PLGA nanoparticles with PC as an organic phase solvent, when DMAB, PVA and Pluronic F68 are used as stabilizers, respectively. The mean particle sizes for PVA and Pluronic F68 were found to decrease sharply, but little change was observed for DMAB with increasing stabilizer concentration. This indicates that with increasing stabilizer concentration for PVA and Pluronic F68, more stabilizer molecules are adsorbed on the interfaces of emulsion droplets, providing increased protection against coalescence and

Table 2
Summary of mean particle sizes (nm) as a function of stabilizers and organic phase solvents used

	DMAB	PVA	Pluronic
PC	50 ± 2	159 ± 3	213 ± 5
EA	67 ± 3	213 ± 5	117 ± 3
ACE	298 ± 6	214 ± 5	221 ± 5
DCM	390 ± 10	284 ± 6	461 ± 10

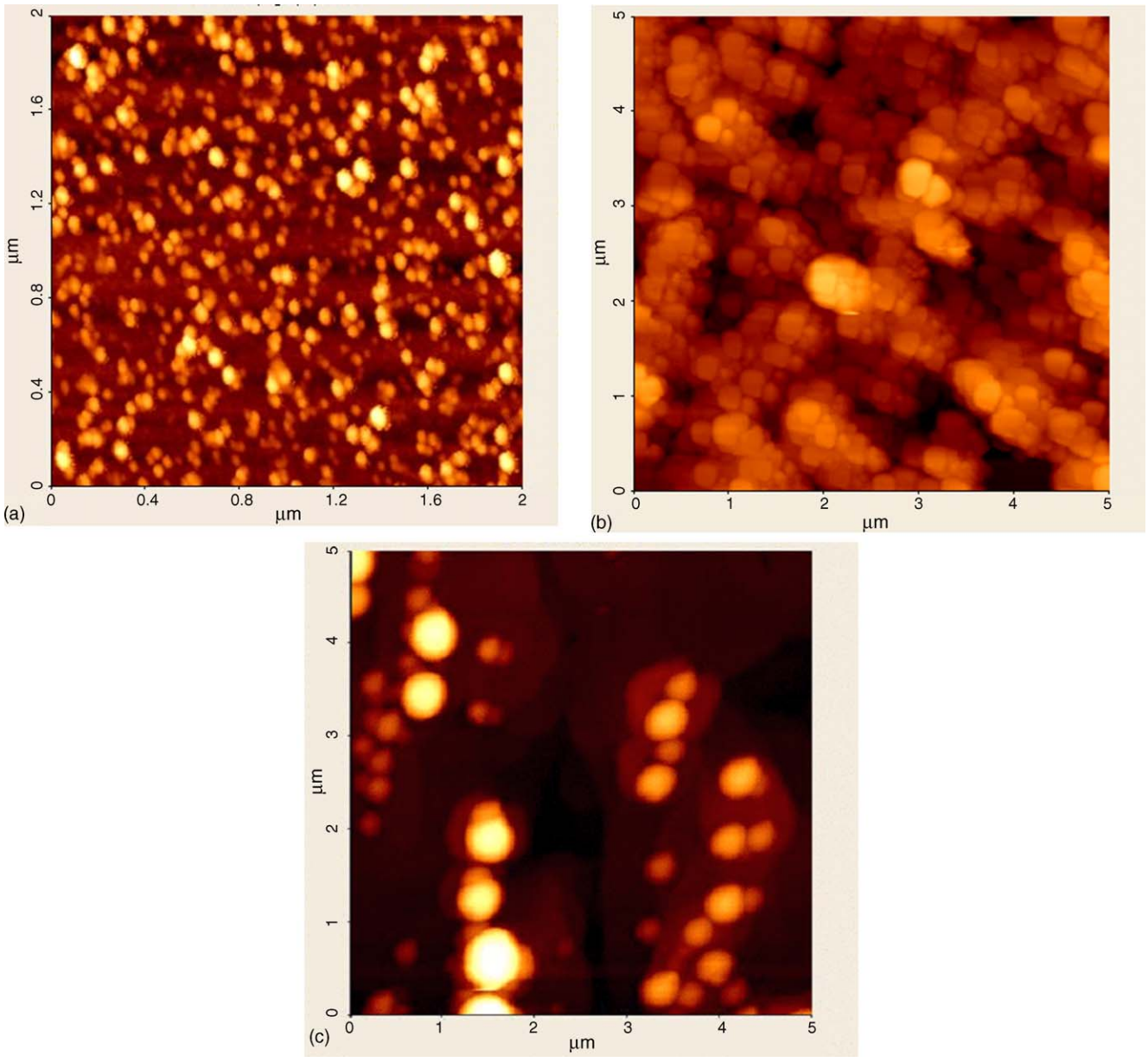

Fig. 2. AFM images of the PLGA nanoparticles prepared with (a) EA, (b) DCM, (c) ACE as organic phase solvents, respectively, when DMAB was used as a stabilizer.

resulting in smaller emulsion droplets. Ahlin et al. [12] prepared poly(methylmethacrylate) (PMMA) nanoparticles using PVA as a stabilizer by the emulsification-diffusion method, and investigated the effect of PVA concentration on the PMMA nanoparticle size. They observed that physical stability of PMMA nanoparticles increased with increasing PVA concentration, leading to smaller nanoparticle size. However, for DMAB, only a small quantity of stabilizer is adsorbed at the interface of emulsion droplets. The excess remains in the continuous aqueous phase and does not play any significant role in the emulsification [13]. The mean size of PLGA nanoparticles prepared using DMAB as a stabilizer is smaller than those of PVA and Pluronic F68, which is in agreement with the result of Kwon et al. [6].

Fig. 6 shows the mean particle sizes of PLGA nanoparticles prepared using different organic solvents, i.e. EA, PC, and ACE as a function of the volume of water addition, when DMAB was used as a stabilizer. When EA and PC, partially water-soluble solvents, were used, the mean particle size decreased rapidly when increasing the volume of water addition in the 20–40 ml range, but little change was observed above 80 ml. However, for ACE, a fully water-soluble solvent, the volume of water addition does not play an important role in the mean particle size compared with EA and PC, and the particle size decreases moderately when increasing the volume of water addition. These results mean that the volume of water addition is an important factor in determining the PLGA particle size for partially water-soluble solvents, PC and EA. Also, it is observed that the mean size of PLGA particles prepared using ACE is larger than those of EA and PC, which is in agreement with the result of particle size distributions in Fig. 1.

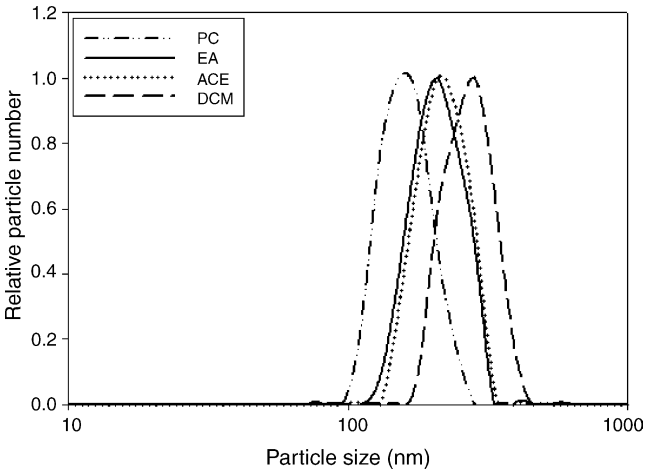

Fig. 3. The effect of different organic phase solvents on the particle size distributions of the PLGA nanoparticles, when PVA was used as a stabilizer.

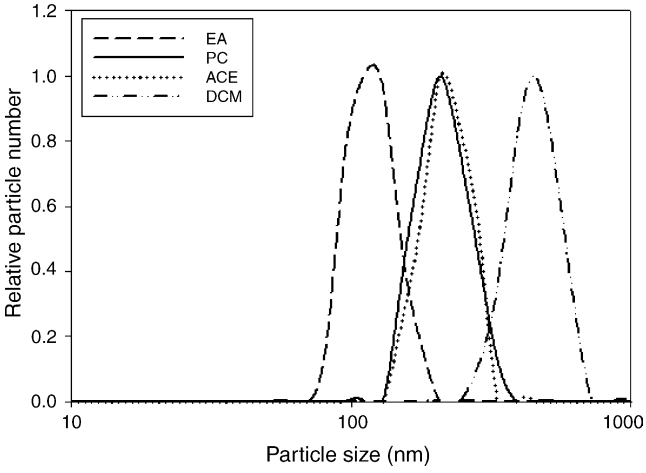

Fig. 4. The effect of different organic phase solvents on the particle size distributions of the PLGA nanoparticles, when Pluronic F68 was used as a stabilizer.

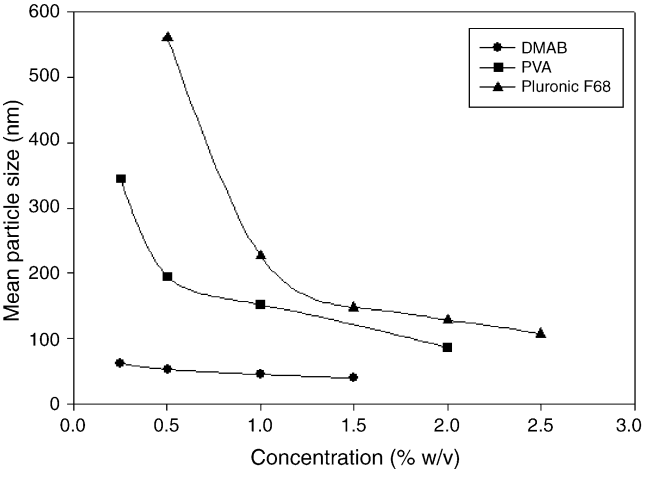

Fig. 5. The effect of the type and concentration of stabilizers on the mean size of PLGA nanoparticles, when PC was used as an organic solvent.

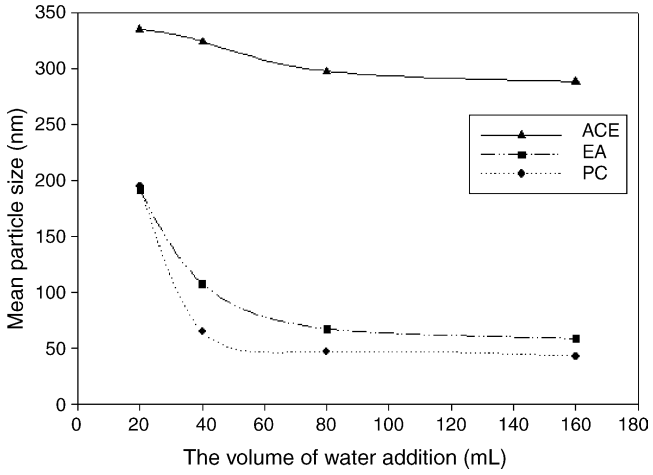

Fig. 6. The effect of the volume of water addition on the mean size of PLGA nanoparticles for different organic phase solvents, when DMAB was used as a stabilizer.

4. Conclusions

The solubility of organic phase solvents in water was an important parameter affecting the mean size of PLGA nanoparticles, when DMAB, an ionic stabilizer, was used. Smaller nanoparticles below 70 nm were obtained using partially water-soluble solvents (EA and PC) than when using a fully water-soluble solvent (ACE) or water-immiscible solvent (DCM). In particular, when PC was used as an organic phase solvent, PLGA nanoparticles below 50 nm were obtained. However, when PVA and Pluronic F68, nonionic stabilizers, were used, a big difference in particle size distributions between partially water-soluble solvent or fully water-soluble solvent and water-immiscible solvent was not observed, and all particles showed a large mean diameter above 110 nm, irrespective of the type of organic solvents.

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