Thermodynamic parameters on poly(D,L-lactide-*co*-glycolide) particle size in emulsification–diffusion process

Sung-Wook Choi, Hye-Young Kwon, Woo-Sik Kim, Jung-Hyun Kim *

Nanosphere Process & Technology Laboratory, Department of Chemical Engineering, Yonsei University, 134 Shinchon-dong, Sudaemoon-ku, Seoul 120-749, South Korea

Abstract

The emulsification–diffusion method was thermodynamically studied for making poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles quantitatively considering the diffusion and the solvent–polymer interaction. The properties of various solvents and polymer were also evaluated on the formation of PLGA nanoparticles, such as diffusion coefficients (D_{sw}, D_{ws}), exchange ratio ($R = D_{sw}/D_{ws}$), and solvent–polymer interaction parameter (χ). R was found to be proportional to the size of the PLGA nanoparticles, while χ was inversely proportional to it. In the case of the higher value of R and lower value of χ, a small local supersaturation region was produced at the O/W interface and the small nanoparticles separated from the oil globule were formed in that region. This thermodynamic approach provides a rational basis for the selection of solvent to control the size of PLGA nanoparticles. © 2002 Elsevier Science B.V. All rights reserved.

Nomenclature

D_p particle size (nm)
D_{sw} diffusion coefficient from solvent to water ($cm^2 s^{-1}$)
D_{ws} diffusion coefficient from water to solvent ($cm^2 s^{-1}$)
e_{coh} cohesive energy density ($J\ cm^{-3}$)
E_{coh} cohesive energy ($J\ mol^{-1}$)
P parachor ($cm^3\ g^{1/4}\ s^{-1/2}\ mol^{-1}$)
R exchange ratio (dimensionless)
T temperature (K)

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* Corresponding author. Tel.: +82-2-2123-4162; fax: +82-2-312-0305.
  E-mail address: jayhkim@yonsei.ac.kr (J.-H. Kim).

V molar volume ($cm^3 \, mol^{-1}$)
δ solubility parameter ($J^{1/2} \, cm^{-3/2}$)
ΔG_M free energy of mixing (J)
ΔH_M enthalpy of mixing (J)
Δh_M enthalpy of mixing per unit volume ($J \, cm^{-3}$)
ΔS_M entropy of mixing ($J \, K^{-1}$)
σ surface tension ($10^{-3} \, N \, m^{-1}$)
η viscosity (cP)
χ solvent–polymer interaction parameter (dimensionless)
ϕ volume fraction

1. Introduction

For the past two decades, lots of work has been conducted to apply biocompatible and biodegradable polymers for drug delivery systems. Among those polymers, much attention has been paid to poly(D,L-lactide-*co*-gylcolide) (PLGA) as drug carriers. Polymeric nanoparticles are widely under investigation as pharmaceutical dosage form of proteins and peptides. Many methods are currently available for the preparation of polymeric nanoparticles, such as emulsification–evaporation method [1], salting-out procedure [2] and nano-precipitation method [3]. Nevertheless, several difficulties have been found for adopting these methods. The usage of solvent may cause toxicity, stabilizers such as polyvinyl alcohol (PVA) cannot be accepted for intravascular usage, and the salts are incompatible with bioactive compound.

The emulsification–diffusion method was recently developed by Niwa et al. [4] and followed by subsequent studies [5–7]. 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 solvent to diffuse into the external phase, resulting in the formation of nanoparticles. Quintanar-Guerrero et al. [5] studied the effects of several preparative variables on nanoparticle size, such as the type and concentration of the stabilizers (poloxamer 188, polysorbate 80, gelatin, polyvinylpyrrolidine and dextran), the stirring speed, the internal/external phase ratio, the polymer concentration in the organic phase, the pH

and the viscosity of external phase. The degrees of hydrolysis and polymerization of PVA were also investigated on the preparation of PLGA nanoparticle [6]. Various solvents were tested on the particle formation, such as ethyl acetate, propylene carbonate and benzyl alcohol [7]. In our previous paper [8], the operational parameters (the type and concentration of stabilizer, homogenizer speed, and polymer concentration) were also investigated for the size of PLGA nanoparticles.

All of these reports have focused on the effects of operational parameters, but there was no thermodynamic approach on the particle formation in the emulsification–diffusion method. In this paper, the influence of the thermodynamic parameters (diffusion coefficients, solubility parameters and solvent–polymer interaction parameters) on the size of PLGA nanoparticle prepared by emulsification–diffusion method was investigated. For the selection of solvents, partially water-soluble solvent should be used, because fully water-soluble solvents often give unsatisfactory results in the preparation of nanoparticles. Therefore, the selected solvents were ethyl acetate (EtAc), methyl ethyl ketone (MEK), propylene carbonate (PC) and benzyl alcohol (BA).

2. Experimental

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) (PLGA) was purchased from Sigma (St. Louis, MO), and its

molecular weight ranges from 75 000 to 120 000, and its copolymer ratio of D,L-lactide to glycolide is 75:25. Poloxamer 188 was obtained from BASF (Pluronic® F68). Methyl ethyl ketone (MEK), ethyl acetate (EtAc), propylene carbonate (PC) and benzyl alcohol (BA) (Junsei Chemical Co., Japan) were used as solvents. Milli-Q water (Millipore, USA-Bedford, MD) was used as distilled and deionized water throughout. All organic solvents were either HPLC grade or American Chemical Society analytical grade reagents.

2.2. Preparation of PLGA nanoparticles

The PLGA nanoparticles were prepared using emulsification–diffusion method. In brief, 200 mg of PLGA was dissolved in 10 ml of solvent (EtAc, MEK, PC, and BA). These organic phases were added into 20 ml of an aqueous phase containing the stabilizer, poloxamer 188. After mutual saturation of organic and continuous phases, the mixture was emulsified for 7 min with a high-speed homogenizer at 12 000 rpm (Omni International Waterbury, CT, USA). For full diffusion into the water phase, 500 ml of water was added to the O/W emulsion under moderate magnetic stirring, leading to the nanoprecipitation of the polymer.

2.3. Particle size analysis

The mean particle diameter of the nanoparticles was measured by the dynamic light scattering method (Zeta plus, Brookhaven Inst. Co., USA). Particle size was expressed as number-weighted mean diameter in nanometers. Measurements were made in triplicate.

3. Results and discussion

3.1. Mechanism for the formation of PLGA nanoparticles

The emulsification–diffusion method has been used successfully to prepare biodegradable nanoparticles in an efficient and reproducible manner. Fig. 1 shows the formation mechanism of PLGA nanoparticles. After the mutual saturation of the two phases (the partially water-soluble solvent containing polymer and water containing stabilizer), both phases were in the state of thermodynamic equilibrium. Homogenization resulted in the dispersion of the solvent as globules, in equilibrium with the continuous phase. The stabilizer was adsorbed on the interfacial area of solvent droplets. The addition of water made the solvent diffuse to the external phase and the supersaturation region was produced at the interface of the O/W emulsion. Therefore, polymer aggregation was provoked in the form of solid colloidal particles and nanoparticle were formed. The characteristic of this technique is the rapid diffusion of solvent from the internal into the external phase.

3.2. The effect of exchange ratio

Diffusion phenomenon is significant for the formation of PLGA nanoparticle in the emulsification–diffusion method. Bodmeier and McGinity [9] established that the rate of polymer precipita-
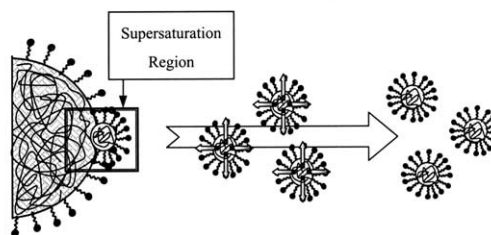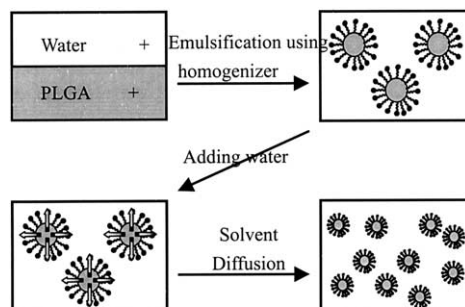

Fig. 1. Mechanism for the formation of PLGA nanoparticles.

tion was significantly affected by the rate of diffusion of the organic solvent into the aqueous phase when microsphere was prepared by the solvent evaporation method. During evaporation of solvent, one polymer particle is formed in each emulsion droplet. Although vigorous agitation of the system by a high-pressure homogenizer was useful to reduce the particle size, it was impossible to produce nanoparticles with diameter less than 1 μm [9].

Niwa et al. [4] formulated acetone into the solvent-evaporation process and found that interfacial tension decreased with increasing amount of acetone in the organic solvent. In the actual preparation process, the concentration gradient of acetone at the interface of the two phases existed even at lower concentrations of acetone in the organic phase. This variation induced interfacial turbulence or spontaneous agitation of the interface between the two unequilibriated liquid phases, which was governed by the so-called Marangoni effect [10]. The diffusion and evaporation of the organic solvents (from the organic dispersed droplets) and the counter-diffusion of water (into the droplets) reduced the solubility of the PLGA and deposited it in the droplets, forming the nanoparticles. In the process of nanoprecipitation [11], nanoparticles were formed due to the mutual diffusion between acetone (solvent) and water. When a polymer solution in acetone was poured or injected into an aqueous stabilizer solution, a violent spreading was observed and droplet of the oil, were torn from the interface. In this process, interfacial phenomena during diffusion contributed to the subdivision of the globule into nanodroplets. The interfacial turbulence was caused by the locally lowered interfacial tension and the molecular mechanism of interfacial turbulence could be explained by the continuous occurrence of solvent diffusion at the interface.

Quintanar-Guerrero et al. [5] proposed 'diffusion-stranding' formation mechanism of nanoparticles by emulsification–diffusion method. The basic idea was that diffusion of partially water-soluble solvent from the globules carried polymer molecules into the aqueous phase, forming local regions of supersaturation from which, new globules or polymer aggregates were formed.
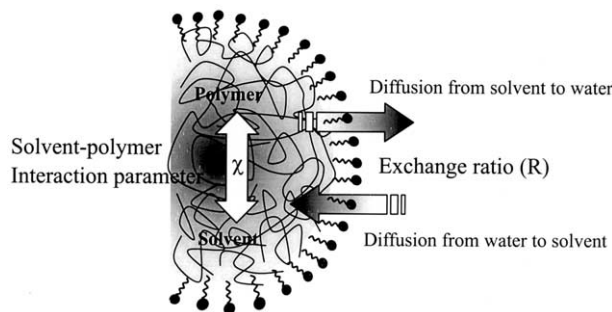

Fig. 2. Schematic illustration for the mutual diffusion and solvent–polymer interaction.

This diffusion motion is closely related to the solvent–non-solvent–polymer interactions. Organic solvents of low water solubility (e.g. chloroform) result in slow precipitation and microparticles will be formed by the solvent loss from the surface of the droplet. Therefore, the diffusion motion of the solvent plays an important role in explaining the variation of the PLGA nanoparticle size.

As shown in Fig. 2, diffusion occurs from the solvent phase to the water phase as well as from the water phase to the solvent phase. It may be assumed that D_{sw} is the important factor rather than D_{ws}. However, D_{ws} has to be taken into account because the size of resulting particles was measured in the suspension state. Therefore, the mutual diffusion coefficients (D_{AB} and D_{BA}) have to be taken into account.

Several estimation methods have been developed to predict mutual diffusion coefficients. Among the various methods [12], Tyn and Calus have proposed that D^0_{AB} be estimated by the following relation:

$$D^0_{AB} = 8.93 \times 10^{-8}\left(\frac{V_A}{V_B^2}\right)^{1/6}\left(\frac{P_B}{P_A}\right)^{0.6}\frac{T}{\eta_B}, \qquad (1)$$

where D^0_{AB} (cm^2 s^{-1}) is mutual diffusion coefficient of A in B, T (K) is the temperature, η_B (cP) is the viscosity of solvent B, V_A (cm^3 mol^{-1}) is the molar volume of A at its normal boiling temperature, V_B (cm^3 mol^{-1}) is the molar volume of B at the normal boiling temperature, and P_A, P_B are parachors for A and B, respectively. This equa-

tion was used to calculate the mutual diffusion coefficients.

The term of exchange ratio (R) was defined as follows:

$$\text{exchange ratio } (R) = \frac{D_{sw}}{D_{ws}} =$$

$$\frac{\text{Diffusion from solvent to water}}{\text{Diffusion from water to solvent}}. \qquad (2)$$

The calculated mutual diffusion coefficient and exchange ratio of various solvents are shown in Table 1 and Fig. 3, respectively. It was found that the particle size was increased with the increase of the exchange ratio and that tendency was roughly exponential. Although MEK has the largest D_{sw} and D_{ws} among the solvents used, the PLGA particle size using EtAc is smaller than with other solvents. It can be assumed that as the exchange ratio is large, the smaller supersaturation region is produced and polymers in that region aggregates. Therefore, smaller particles would be obtained when the higher exchange ratio produced the smaller local supersaturation region near the interface.

3.3. The effect of solvent–polymer interaction parameter

Quintanar-Guerrero et al. [13] suggested that the mechanism of the nanoparticle formation by this method was due to diffusion alone. However, the diffusion motion is associated with the mutual relations of the polymer–non-solvent–solvent system. The effect of the interaction between solvent and water was discussed in Section 3.2 with re-
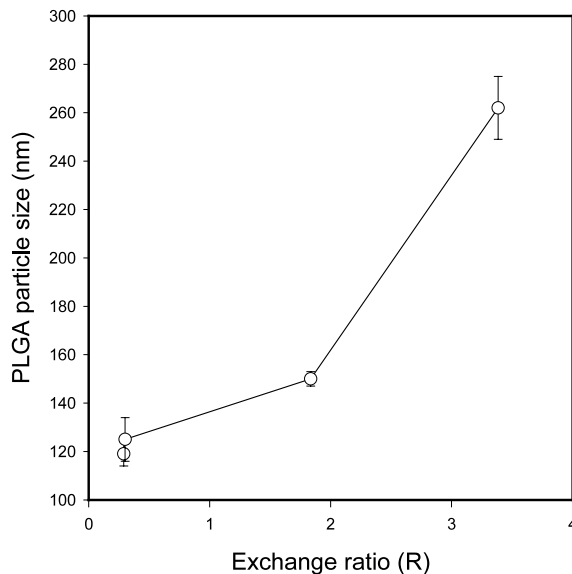

Fig. 3. Effect of exchange ratio on PLGA particle size.

spect to the mutual diffusion coefficients. Therefore, in this section the effect of the affinity between polymer and solvent on the PLGA particle size will be discussed.

Solubility parameters are the most used quantities for the selection of solvent, to predict their compatibility with some substance such as polymers and pigments. The solubility parameter, δ, defined by Hildebrand [14] as the square root of the cohesive energy density:

$$\delta = \left(\frac{E_{coh}}{V}\right)^{1/2} = (e_{coh})^{1/2} \qquad (3)$$

where E_{coh} is the energy of vaporization and V, the molar volume is a measure of all the intermolecular forces, responsible for the material cohesion.

To estimate the solubility parameter, many methods have been proposed, for example by Hayes, Small, Hoy and Hoftyzer and Van Krevelen [14]. The solvent–polymer interaction is generally affected by many factors such as the molecular weight of polymer, the chemical structure, the molar volume of solvent and the temperature. In this work, the average value calculated

Table 1
Calculated mutual diffusion coefficient of various solvents

Solvent	$D_{\text{solvent to water}}$	$D_{\text{water to solvent}}$	Exchange ratio (R)
BA	9.699×10^{-6}	2.865×10^{-6}	3.385
PC	1.045×10^{-5}	5.685×10^{-6}	1.838
MEK	1.082×10^{-5}	3.588×10^{-5}	0.301
EtAc	1.053×10^{-5}	3.541×10^{-5}	0.297

BA, benzyl alcohol; PC, propylene carbonate; MEK, methyl ethyl ketone; EtAc, ethyl acetate.

by the above-mentioned methods (Table 2) was used to estimate solubility parameter of the polymer and solvents. Solvent–polymer interaction parameter (χ) can be written in terms of solubility parameter as:

$$\chi = \frac{V_{\text{solvent}}}{RT}(\delta_{\text{solvent}} - \delta_{\text{polymer}})^2 \qquad (4)$$

where V_{solvent} is the molar volume of the solvent and R is the gas constant.

According to Hildebrand [14], the enthalpy of mixing can be calculated by

$$\Delta h_M = \phi_1\phi_2(\delta_1 - \delta_2)^2 \qquad (5)$$

where, Δh_M is the enthalpy of mixing per unit volume, ϕ_1 and ϕ_2 are the volume fractions of components 1 and 2, espectively, and δ_1 and δ_2 are the solubility parameters of components 1 and 2, respectively.

From the above equation, it can be assumed that if $\delta_1 = \delta_2$, two substances with equal solubility parameters should be mutually soluble due to the negative entropy factor. This is in accordance with the general rule that chemical and structural similarity favors solubility. As the difference of the solubility parameters increases, the tendency towards dissolution decreases. It may be concluded that as a requirement for the solubility of a polymer P in a solvent S, the quantity $(\delta_P - \delta_S)^2$ has to be small, as small as possible.

The influence of solvent–polymer interaction parameter on particle size is shown in Fig. 4. With increase in the solvent–polymer interaction parameter, the particle size decreases. The quan-
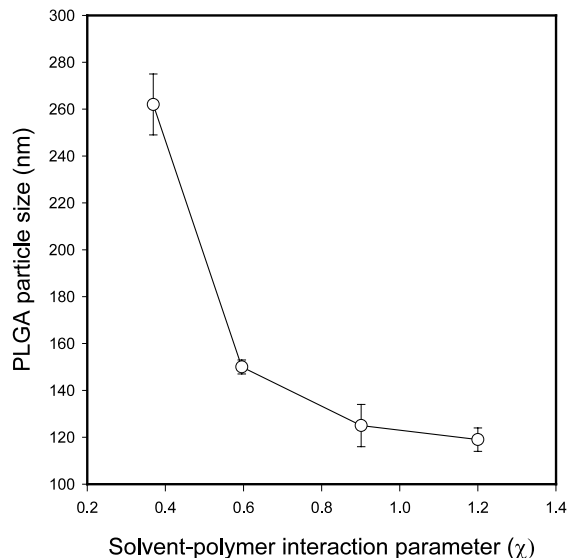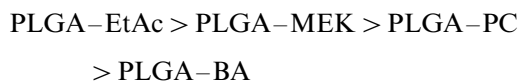

Fig. 4. Effect of solvent–polymer interaction parameter on PLGA particle size.

tity $(\delta_P - \delta_S)^2$ shows the following order:

$$\text{PLGA–EtAc} > \text{PLGA–MEK} > \text{PLGA–PC}$$
$$> \text{PLGA–BA}$$

The quantity $(\delta_P - \delta_S)^2$ shows the degree of affinity between the solvent and the polymer. As $(\delta_P - \delta_S)^2$ increases, the degree of affinity between the polymer and the solvent decreases. It could be assumed that when the affinity between the solvent and the polymer was higher, the larger regional supersaturation spot was produced. Therefore, the large nanoparticles were formed.

With these results, the mechanism of the emulsification–diffusion method can be explained by a combination of solvent–water exchange effect and polymer–solvent interaction effect.

4. Conclusions

This study has shown the effect of solvent properties on the PLGA particle size in the emulsification–diffusion method. It was elucidated that the size of PLGA nanoparticle was influenced

Table 2
Calculated solubility parameters and solvent–polymer interaction parameters of various substances

Solvent	Average solubility parameter (δ)	Polymer–solvent interaction parameter (χ)
BA	22.40	0.369
PC	25.97	0.595
MEK	19.30	0.901
EtAc	18.57	1.200

Average solubility parameter of PLGA = 23.24. BA, benzyl alcohol; PC, propylene carbonate; MEK, methyl ethyl ketone; EtAc, ethyl acetate.

by the thermodynamic parameters such as mutual diffusion coefficients and solvent–polymer parameters. The size of PLGA nanoparticle was decreased with the decrease of exchange ratio (R) and the increase of the solvent–polymer interaction parameter (χ). As the solvent had the high R and low χ, a small supersaturation region was produced and therefore small nanoparticle could be obtained. These results would be helpful for the solvent selection in emulsification–diffusion method and for the prediction of PLGA nanoparticle size.

Acknowledgements

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