The Effect of Process Parameters on the Size and Morphology of Poly(D,L-Lactide-*Co*-Glycolide) Micro/Nanoparticles Prepared by an Oil in Oil Emulsion/Solvent Evaporation Technique

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ABSTRACT: For the past few decades, there has been a considerable research interest in the area of biodegradable polymeric micro- and nanoparticles for tissue engineering, regenerative medicine, implants, stents, medical devices, and drug delivery systems. Poly(D,L-lactide-*co*-glycolide) (PLGA) is well-known by its safety in biomedical preparations which has been approved for human use by the FDA. The goal of this study was to evaluate the influence of process parameters on size characteristics of PLGA microparticles prepared by oil in oil (o/o) solvent evaporation technique. This method has been introduced as one of the most appropriate methods for hydrophilic agents. Scanning electron microscopy showed that prepared particles were spherical with smooth surface without aggregation. Particle size varied from 570 nm to 29 μm in different experimental conditions. Stirring speed, polymer concentration, impeller type, and dropping size had a significant effect on the particle size. The polydispersity index of particles showed a strong relationship with the surfactant concentration, impeller type, and dropping size. It was concluded that increasing in temperature up to 50°C or changing in dropping rate has a little effect on reducing the size of PLGA particles. The residual solvent content in the final suspension was less than 0.1 ppm that is in appropriate range for biomedical application. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 528–534, 2010

Key words: PLGA; nanoparticles; microparticles; O/O emulsion/solvent evaporation

INTRODUCTION

Biodegradable micro- and nanoparticles can be useful delivery devices for various active agents.[1] Poly(D,L-lactide-*co*-glycolide) is one of the most common biodegradable and biocompatible polymers used for the controlled delivery of active agents[2,3] which has been approved for human use by the FDA.[4] Emulsion/solvent evaporation technique has been chosen as one of the frequently used methods for preparation of PLGA micro- and nanoparticles.[5,6] Particle size, size distribution, morphology and residual solvent are key parameters in the formulation of micro/nanoparticles. Size and structure of particles influence the therapeutic efficacy, release kinetics and other pharmaceutical and cellular uptake features of the particulate delivery system.[7,8] It is also found that the possibility of particle endocytosis depends on their size.[9,10] Interaction with the mucous membranes is also partly determined by particle size.[11,12] Extensive and various studies have been advanced to achieve the optimized carrier size and surface characteristics for extended blood circulation of carrier and effective delivery of drug to the target sites.[13–15] To reach the lower respiratory tract and optimize systemic

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TABLE I
Investigated Process Parameters

Batch No.	Polymer con.[a] (w/v %)	Stirring speed (rpm)	Surfactant con.[a] (v/v %)	Temperature (°C)	Dropping Rate (min)	Nozzle orifice (μm)	Impeller type
1	1.25	2000	0.50	50	30	170	Sawtooth
2	2.5	2000	0.50	50	30	170	Sawtooth
3	5	2000	0.50	50	30	170	Sawtooth
4	10	2000	0.50	50	30	170	Sawtooth
5	20	2000	0.50	50	30	170	Sawtooth
6	20	2000	0.25	50	30	170	Sawtooth
7	1.25	3000	0.50	50	30	170	Sawtooth
8	1.25	5000	0.50	50	30	170	Sawtooth
9	1.25	5000	0.50	RT[b]	30	170	Sawtooth
10	2.5	2000	0.50	50	30	1000	Sawtooth
11	2.5	2000	0.50	50	0.5	170	Sawtooth
12	2.5	2000	0.50	50	30	170	Rushton

[a] Polymer and surfactant concentrations.
[b] Room temperature.

drug absorption, inhalation aerosols need to present diameters between 1 and 5 μm.[16–18] Larger particles impact in the oro-pharynx while submicron particles remain suspended in air and are exhaled.[19] Residual of organic solvents such as Acetonitrile, *n*-Hexane, Methylene chloride and Chloroform used frequently in emulsion solvent evaporation method can cause some problems. These residual solvent impurities in the polymer can lead to reduction in the efficiency of the pharmaceutical product, increased toxicity, undesirable side effects, and other complications, creating significant regulatory hurdles in the product development. It is difficult to remove these solvents quantitatively, therefore traces of them remain in the micro/nanospheres.[20–22] Since the o/o emulsion/solvent evaporation technique was used successfully by our group for encapsulation of proteins such as insulin,[23] erythropoietin and virus (unpublished data), the knowledge about the effect of process parameters on the size range of PLGA particles prepared by this method for various delivery applications would be desirable. According to our literature review, the effect of process parameters on the size characteristics and the evaluation of residual solvents in the particles have not been investigated in the oil/oil (o/o) emulsification method. Therefore, the objective of this study is to investigate the influence of process parameters including polymer and surfactant concentrations, temperature, stirring speed, dropping rate, droplet size, and stirrer paddle type on the morphology, polydispersity and particle size of poly(D,L-lactide-*co*-glycolide) micro/nanoparticles. We also determined the residual solvents in the PLGA particles made by this fabrication process.

MATERIALS AND METHODS

Materials

PLGA (RG504H, lot 1020751) was supplied by Boehringer Ingelheim, Germany. The molar ratio of glycolic acid to L-lactic acid in PLGA was 50 : 50. Span 80® (Sorbitan monooleate) was purchased from Fluka (Switzerland). Acetonitrile, *n*-Hexane and viscous mineral oil were obtained from Merck (Germany). Filter membrane (PTFE) having a pore size of 0.22 μm purchased from Sartorius AG (Germany). All other reagents were analytical or reagent grade and used as received.

Methods

Preparation of micro/nanoparticles

PLGA micro/nanospheres were prepared by an o/o emulsion/solvent evaporation technique. The polymer solution (3 mL) was prepared by dissolving the polymer in Acetonitrile. This solution was added into viscous liquid paraffin (40 mL in 100 mL beaker) containing Span 80 heated and stirred for 2 h to ensure complete evaporation of Acetonitrile. Micro/nanospheres were collected by centrifugation (Sigma 3K30, Rotor No. 12150, Germany) at 20,000 rpm for 30 min at 10°C and washed four times with *n*-Hexane to remove mineral oil. Particles were filtered, vacuum dried and stored under refrigeration in a desiccators until used. Different formulations were designed to evaluate the effect of process parameters on the size and size distribution of prepared particles and yield of procedure (Table I).

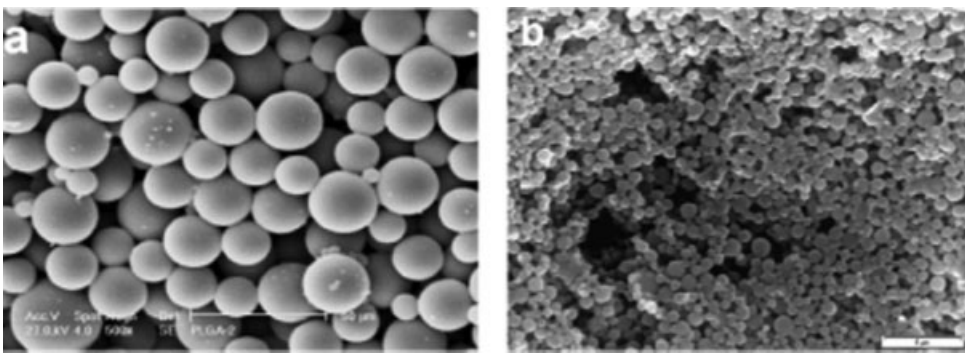

Figure 1 Scanning electron microscopy images showing the external surface structure of PLGA microspheres. a) batch No. 5 (×500, scale bar 50 μm), b) batch No. 1 (×4000, scale bar 1 μm).

Characterization

Morphology. Scanning electron microscopy (SEM) was used to determine the surface morphology of the particles (Philips, XL30, The Netherlands). Upon preparation, particles were placed on a double stick tape over aluminum stubs to get a uniform layer of particles. Samples were gold-coated using a sputter gold coater (BAL-TEC, Switzerland) at 40 mA current and 10^{-3} Torr pressure for 400 s at a thickness of 400 Å.

Atomic force microscopy (AFM) was used for a more accurate surface analysis of microspheres. The surface topography of the microcapsules were investigated by using a Dualscope controller, AFM and scanner (DME, Denmark) in noncontact Mode operation (AC-AFM). Tetrahedral-tipped silicon cantilevers (MicroMash) with a nominal tip radius of curvature <10 nm, force constant 42 N/m and a resonant frequency 200–400 kHz were utilized for imaging.

Particle size analysis. The particle size was determined by laser light scattering (Sematech, French for nano particles and Malvern Mastersizer X, UK suitable for microparticles). About 5 mg of the sample was dispersed in 5 mL of water containing 0.1% Tween 80 and sonicated (Starsonic 60, Liarre, Italy) for 2 min. The size and size distribution were expressed in terms of Z average for nanoparticles and the volume mean diameter (VMD) for microparticles and span, respectively. Span, index of polydispersity, is calculated using the following equation:

$$\text{Span} = \frac{D(v,90) - D(v,10)}{D(v,50)}$$

where $D(v,90)$, $D(v,10)$, and $D(v,50)$ are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively.

Yield of Particles. The production yield was calculated by the following equation:

$$\text{Yield} = \frac{W_2 - W_1}{W_1} \times 100$$

where W_1 was the initial weight of polymer and W_2 was the weight of particles collected on the surface of filter at the end of procedure.

Measurement of solvent residual. Residual solvents were analyzed using Agilent GC6890/MSD5973 (Agilent Technologies). Particles were dissolved in *N*-methylpyrrolidone in a sealed vial to release all entrapped residual solvent.

Sample was directly injected into a AP-MS column (30m in length).

RESULTS AND DISCUSSION

Morphology of microspheres

The scanning electron microphotographs (SEM) of microspheres are shown in Figure 1. It was found that uniform PLGA microspheres were successfully prepared by the o/o solvent evaporation method. As shown in Figure 1, the PLGA microspheres were spherical and had a smooth surface without pores or cavities. The microspheres were relatively uniform without agglomeration. All formulations prepared under various conditions possessed similar properties with regard to shape and appearance. All narrow-sized, spherical particle powders with a span less of than 2 was obtained. For further investigation of the morphological properties, AFM whose resolution is far beyond the capacity of the SEM was used. The representative AFM images of the flat and smooth surface of microspheres are shown in Figure 2.

The effect of process parameters on the characteristics of PLGA microspheres

The size, size distribution and yield of PLGA microparticles prepared by the o/o solvent evaporation

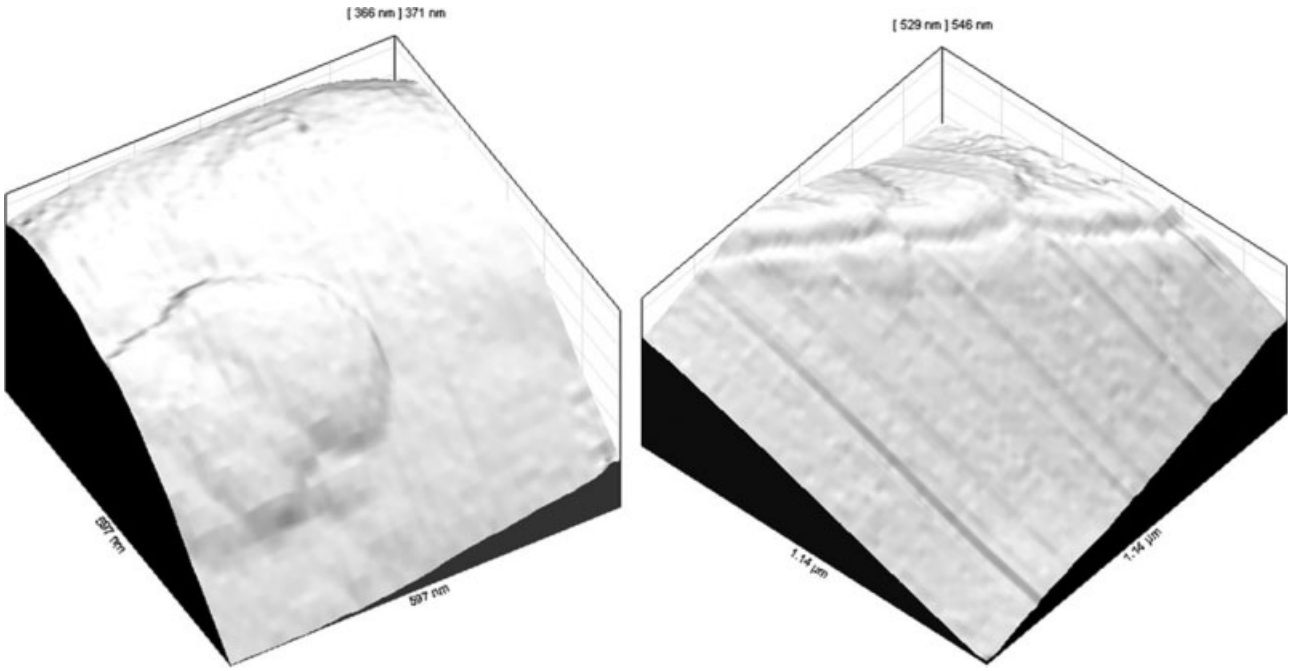

Figure 2 Atomic force microscopy images of PLGA particles (Left Fig., 597 nm × 597 nm; Right Fig., 1.14 µm × 1.14 µm).

technique under various process conditions such as temperature, stirring speed, impeller type, dropping rate, droplet size, polymer and surfactant concentration was reported in Table II.

The effect of polymer concentration

The influence of the initial concentration of PLGA in Acetonitrile at 1.25, 2.5, 5, 10, and 20 (w/v %) on the size distribution of the particles (formulations No. 1–5, Table I) are summarized in Table II. By increasing the PLGA concentration from 1.25 to 20% w/v, the size of particles increased from 3.5 to 20.65 µm which is consistent with the previous reports.[24–27] Increase of particle size might have been due to an increase in viscosity of polymer solution which hinders the homogenous dispersion of PLGA solution into the oil phase. Polydispersity had no meaningful relationship with PLGA concentration. As illustrated in Table II, particles prepared in upper and lower extremes of PLGA concentrations showed more uniform size distributions.

Surfactant concentration

The amount of surfactant plays an important role in the emulsification process and the protection of the droplets, because of avoiding the coalescence of globules.[28,29] Comparing the formulations No. 5 and 6 exhibits the effect of different surfactant concentrations on particle size and polydispersity. By increasing the surfactant concentrations, the interface between internal (polymer in Acetonitrile) and external (liquid paraffin oil) phase will be covered more. Therefore the droplets will be better protected against coalescence, consequently it leads to the formation of smaller emulsion droplets. Polydispersity index revealed that the distribution of particles were more uniform as the amount of surfactant was increased. It might be interpreted by stability of emulsion during particle formation.

Stirring speed

Stirring is the most simple way to generate droplets of the polymeric dispersion in the continuous extraction phase for subsequent solvent removal. The stirring speed of the emulsion apparatus is a critical

TABLE II
Obtained Experimental Responses for Different Formulations (Each Number Represents Mean ± Standard Deviation, $n = 3$)

Batch No.	Size (µm)	Polydispersity	Yield (%)
1	3.20 ± 1.2	1.09 ± 0.05	79.7 ± 3
2	4.01 ± 1.5	1.10 ± 0.06	75.5 ± 7
3	4.53 ± 1.8	1.48 ± 0.09	70.8 ± 4
4	10.2 ± 1.5	1.19 ± 0.07	68.5 ± 3
5	20.6 ± 3.5	0.90 ± 0.02	70.7 ± 4
6	28.8 ± 3.1	1.34 ± 0.08	71.4 ± 3
7	0.89 ± 0.4	1.12 ± 0.06	45.3 ± 4
8	0.64 ± 0.2	1.21 ± 0.08	39.5 ± 8
9	0.57 ± 0.1	1.14 ± 0.07	38.5 ± 9
10	6.81 ± 2.8	1.20 ± 0.08	78.9 ± 5
11	4.23 ± 1.5	1.14 ± 0.06	75.5 ± 7
12	7.82 ± 1.2	1.51 ± 0.09	75.4 ± 6

determinant of the particles size.[30] Increasing the stirring speed generally results in a decrease in the mean particle size,[2,31–34] as it produces smaller emulsion droplets through stronger shear forces and increased turbulence. In the present study, stirrer speeds of 2000 (mechanical stirrer, IKA ABORTECHNIK, Model RW 20DZM), 3000 and 5000 rpm (mechanical stirrer, Tohid Sanat Sepahan, Model TSS55) were used to disperse the internal phase (PLGA dissolved in acetonitrile) in continuous phase (40 ml paraffin oil containing 0.5% Span 80). As indicated in Table II, increasing the stirring speed from 2000 to 5000 rpm (formulations No. 1, 7 and 8) led to a decrease in the size of PLGA particles from 3.5 μm to 640 nm. In addition, the narrower size distributions of PLGA particles (smaller polydispersity) were observed when lower stirring speed used (Table II).

Temperature

The rate of volatile solvent removal from the solidifying microspheres can be controlled by the temperature of the microsphere dispersion. Higher temperatures will facilitate the evaporation of the solvent from the continuous phase and thereby maintain a high concentration gradient for the solvent between the microspheres and the continuous phase.[34] Although the difference in size values were not statistically significant ($P > 0.05$; independent t-test) comparison of formulations 8 and 9 reveals that particle size increased slightly by an increase in the temperature which may be attributed to very rapid formation of the particles. High temperatures led to an increase in evaporation rate of Acetonitrile (PLGA solvent) and rapid formation of the particles. The PLGA particles tended to be larger with more uniform size distributions when prepared at higher temperatures (i.e. 50°C).

Droplet size

The effect of nozzle orifice diameter, which influences the diameters of internal phase droplets, on the size and polydispersity of the particles was also investigated. To accomplish this Acetonitrile PLGA solution was poured into the oil phase through two nozzles, burette and 27 G needle with the orifice of 1 mm and 0.17 mm, respectively. As summarized in Table II, the results of formulations No. 2 and 10 showed that increasing the nozzle orifice diameter from 0.17 mm (formulation 2) to 1 mm (formulation 10) led to an increase in the size of PLGA particles from 4.2 to 6.8 μm. It was also observed that decreasing the initial droplet size in oil phase causes smaller particles. The size distribution of microspheres decreased with smaller droplet size.

Dropping rate

The polymer solution was added, dropwise in 30 min (formulation 2) and in 30 s (formulation 11) by 27 G needle, under vigorous stirring the oil phase. The results showed that increasing in dropping rate had no significant effect on the size and polydispersity of PLGA particles ($P > 0.05$, Table II).

Impeller type and shear rate

Sawtooth impeller and 8-blade impeller were used to produce the emulsion. Prepared particles showed dramatic difference in size and polydispersity index. It is concluded that particle size decreased significantly ($P < 0.05$) when Sawtooth impeller was used (formulations No. 2 and 12). Moreover using the Sawtooth impeller led to narrower particle size distributions. The Sawtooth impeller generates a higher shear force and is more efficient for emulsification and dispersion compared to the 8-blade impeller. The observed shear force differences are most likely due to one of the following reasons: The Reynolds number is the ratio of inertial forces to viscous forces and is used for determining whether a flow will be laminar or turbulent. Laminar flow occurs at low Reynolds number, where viscous forces are dominant; this flow is characterized by smooth, constant fluid motion, while turbulent flow occurs at high Reynolds number and is dominated by inertial forces, producing random eddies, vertices and other flow fluctuations.[20] The Reynolds Number is calculated from the following equation:

$$\mathrm{Re} = \frac{D \times G}{\mu}$$

where G, D, and μ are the rotational speed, impeller diameter, and fluid kinematics viscosity, respectively. In a same stirring speed, both rotational speed and kinematics viscosity are constant according to above mentioned equation, increasing the impeller diameter (from 28 to 58 mm) results in stronger shear forces and increased turbulence. As a result, smaller droplets are generated in the emulsion using the impeller with larger diameter. If even the diameter of impellers is equal, 8-blade impellers need a baffle to produce effective shearing force while Sawtooth impellers without baffles can produce a high shear force to prepare a uniform emulsion.[35]

Yield of particles

The yield of particles varied with the stirrer speed. Since for laboratory scale production often small amounts of microparticles in the milligram range are

needed, one advantage of the O/O technique is the high yield of above 70%. The yield of nanoparticles obtained by this method is usually lower (about 40%). To reach necessary turbulence, all of the experiments were carried out in 100 mL flasks. Increasing the stirrer speed caused some of the emulsion containing particles to be spilled from the flask, leading to a loss of nanoparticles.

Measurement of solvent residual

The quality of the products is very strictly regulated in pharmaceutical industry so the qualitative and quantitative knowledge of the residual solvent impurities in the bulk materials and in the final products is essential. The volatile organic solvents used in the manufacture of pharmaceutical formulations need to be removed from the finished product because of their possible health risk and toxicity. Removal of residual solvents to very low concentrations is also important to ensure a safe and stable micro/nanoparticle product.[36] Residual solvents in the pharmaceutical products are classified as three main classes: solvents known to cause unacceptable toxicities which should be avoided in the production of pharmaceutical substances (class 1), solvents associated with less severe toxicities which should be limited in use (class 2) and less toxic solvents (class 3).[37] Acetonitrile and n-Hexane used in this study are classified as class 2 solvents which are allowed to be used in pharmaceutical products but their amount should be limited and accurately determined. The most frequently used technique for the determination of residual solvents in pharmaceutical quality control is gas chromatography (GC). The GC spectrum of the prepared microparticles showed no chromatographic peak. Therefore, the residual solvent concentrations in the product must be less than the detectable limit of the GC-Mass equipment. To confirm the small amount of residual solvents in the product, different concentrations (0.1, 0.25, and 0.5 ppm) of Acetonitrile and *n*-Hexane in *N*-methylpyrrolidone were prepared. No chromatographic peak was observed for samples with concentrations of 0.1 ppm. Consequently, detection limit of this instrument is for concentrations above 0.1 ppm. Acetonitrile and *n*-Hexane residues in the micro/nanoparticles were found to be below 0.1 ppm, which meets the requirements stated in the United State Pharmacopoeia of 410 and 290 ppm, respectively.[37]

CONCLUSION

In this work, O/O emulsion/solvent evaporation was successfully employed for the preparation of biodegradable PLGA micro/nanoparticles. The prepared PLGA micro/nanoparticles presented a spherical morphology with a smooth surface. The polymer and surfactant concentration, stirring speed, impeller type and dropping size had major influence on the mean particle size of PLGA micro/nanoparticles. Dropping rate and temperature had a negligible effect on the size of PLGA particles. In addition, the prepared microparticles met the pharmacopeias requirements in terms of the solvent and residual content.

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