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Fabrication and Surface Characterization of Electrosprayed Poly(L-lactide) Microspheres

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ABSTRACT: Electrospraying is a one-step technique for fabricating polymeric microspheres/nanospheres, and the surface characterization of polymeric microspheres fabricated under high voltage is different from an emulsion method. In this study, biodegradable poly(L-lactide) (PLLA) microspheres were successfully fabricated by electrospraying, and electrospraying parameters were used to investigate the size and ζ potential of the electrosprayed PLLA microspheres. The results demonstrate that electrospraying was a one-step method for fabricating monodispersed PLLA microspheres with a size of 1.92 $\pm$ 0.35 μm and that the enrichment of methyl groups on the surface of the microspheres contributed to the strong hydrophobicity of electrosprayed PLLA microspheres. Of all the electrospraying parameters investigated, the size and ζ potential of the PLLA microspheres increased with increasing solution concentration and flow rate and decreased with increasing injection voltage and collecting distance. The results provide a theoretical basis for preparing electrosprayed polymeric microspheres as drug carriers. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: biodegradable; biomaterials; drug-delivery systems; surfaces and interfaces

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INTRODUCTION

With the rapid development of nanotechnology, polymeric microspheres fabricated by the electrospraying method have drawn more and more attention from researchers. Electrospraying is a one-step technique that atomizes charged polymeric liquid droplets and breaks them up into microspheres.[1] The electrospraying technique is an easy way for preparing microspheres with advantages of good microsphere-forming properties, protection of drug bioactivity, and reduction of the loss of drugs, and this technique has been preliminary applied in the field of biomedicine.[2–4] The surface characterization of polymeric microspheres is the main factor that determines their applications. In the field of biomedicine, the particle size (size and size distribution) and surface characterization (charge and hydrophobicity) of the microspheres influence on the cell's adsorption and phagocytosis and also determine the microspheres' cardiac–vascular cycle, circulation rate, target binding capacity, stability, and drug-release rate.[5] Thus, systematic research of the surface characterization of polymeric microspheres has the value of enlarging their application.

The electrospraying technique is a one-step method for fabricating microspheres with a high drug-loading capacity and controllable morphology and size.[6–8] When compared to the emulsion method, electrospraying has two prominent advantages: the omission of the second emulsion and the avoidance of high temperature; this prevents the introduction of impurities and the inactivation of easy denatured drugs, such as proteins and DNAs.[9–12] This technique has been used to fabricate drug-loaded microspheres of poly(L-lactide) (PLLA), polyphosphazene, poly(lactic-*co*-glycolic acid), lactose and chitosan,[2,13–20] and quantum-dot-encoded poly(styrene–acrylate) microspheres.[4] Hayashi et al.[21] investigated the effects of different polymer types and the concentration on the morphology of electrosprayed microspheres and fabricated microspheres with red-blood-cell-like morphology for the application of fluorescence and magnetic resonance imaging. Scholten et al.[22] investigated the electrosprayed microparticle morphology under different carbamazepine (CBZ) concentrations and the breakup mechanism of charged droplets during the electrospraying process. The results indicate that the morphology and size were determined and controlled by the interplay between jet formation, droplet breakup, solvent evaporation, and particle solidification. Arya et al.[17] fabricated chitosan microspheres with electrospraying and prepared chitosan microspheres with different morphologies and sizes by changing the electrospraying parameters. Bock et al.[5] fabricated polycaprolactone with a narrow size distribution and a size of 10–20 μm by changing

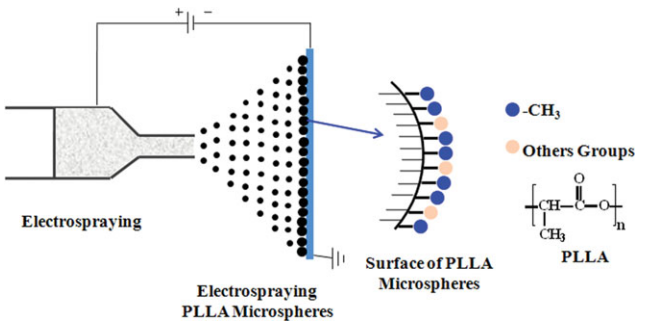

Figure 1. Illustration of the electrospraying process and the chemical group distribution on the surface of the electrosprayed PLLA microspheres. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the electrospraying parameters and demonstrated that the electrospraying parameters could regulate the polymer chain entanglement regime and, by which, regulate the morphology of the microspheres. To date, there have been many reports on the control of the size and morphology of microspheres through changes in the electrospraying parameters, but there have been no reports on the influence of the electrospraying parameters on the surface characterization of microspheres.

In body fluid, the particle size, surface charge, and hydrophobicity of polymeric microspheres are the main factors that influence their circulation and stability.[23–25] During the electrospraying process, the polymer solution rapidly breaks up and atomizes to form microspheres under high voltage; this is different from other microsphere-fabricating methods, and there needs to be systematic research on the surface characterization of microspheres fabricated by electrospraying. In this study, biodegradable PLLA was used to investigate the characterization of electrosprayed microspheres, such as the morphology, size, size distribution, surface distribution of chemical elements, and surface charge, and we systematically investigated the influence of different electrospraying parameters (solution concentration, jet voltage, flow rate, and collecting distance) on the size and ζ potential of the microspheres.

EXPERIMENTAL

Materials

PLLA [weight-average molecular weight (M_w) = 50 kDa, M_w/number-average molecular weight = 1.6] was synthesized in our laboratory. The molecular weight was determined by gel permeation chromatography (Waters 2695 and 2414, Milford, MA, US). Poly(vinyl alcohol) (PVA, M_w = 88 kDa, Jingchun Regents Co., Shanghai, China), ethanol, dichloromethane (DCM), hexafluoroisopropanol (HFIP), and so on were of reagent grade or better and were purchased from Sinopharm Chemical Reagent Co., Ltd.

Electrosprayed PLLA Microspheres

The electrospraying setup and process were performed, as shown in Figure 1. Briefly, PLLA was dissolved in a mixed solvent of ethanol, DCM, and HFIP (ethanol/DCM/HFIP = 2:5:2 w/w/w) at room temperature, and the PLLA solution was added in a syringe pump attached to a high-voltage device. A negative electrode covered with aluminum foil was attached to the ground and used as a collector plate. The electrospraying process was carried out by changes in the PLLA concentration (3–6 wt %), voltage (7–15 kV), collecting distance (10–25 cm), and flow rate (0.02–0.10 mL/min). All of the electrosprayed PLLA microspheres were vacuum-dried at room temperature for 3 days to completely remove any solvent residue before further characterization.

The ordinary PLLA microspheres prepared by a normal oil-in-water emulsion/solvent evaporation method were carried out as described in the literature: PLLA (2.5 wt %) and PVA (2 wt %) were dissolved in DCM and ultrapure water, respectively. The resulting PLLA solution was added dropwise to the PVA solution under vigorous stirring, and the resulting microspheres were washed and freeze-dried to get powder.[26]

Microsphere Characterization

Environmental scanning electron microscopy (SEM; FEI, QUANTA 250, Veldhoven, The Netherlands) was used to investigate the morphology and size homogeneity of the electrosprayed microspheres.

The electrosprayed microspheres were scraped from the aluminum foil and ultrasonically dispersed in PVA solution (0.5 wt %), and the particle size analyzer (Nano ZS, Malvern Instruments, Malvern, UK) was used to measure the size, polydispersity index (PDI), and ζ potential of the electrosprayed microspheres. PDI indicates the size distribution within a particle population.

X-ray photoelectron spectroscopy (XPS; XSAM800, Kratos, Ltd., Manchester, Britain) was used to determine the chemical compositions on the surface of the PLLA microspheres fabricated by the electrospraying method and emulsion method with Mg Kα-1,2 radiation. A detailed analysis of C1s regions was recorded over the binding energy range of 275.0–300.0 eV with a pass energy of 150.0 eV, and data were processed with Kratos Vision 2000. Charging referencing was performed with a C—H peak (285.0 eV), and the overlapping peaks were resolved by the peak synthesis method, with a Gaussian peak component applied after Shirly-type background subtraction.

The membranes formed by the electrosprayed microspheres were attached to slides with double-sided adhesive. The water contact angles (WCAs) on the electrosprayed microspheres surface were measured by contact angle measurement (Krüss GmbH DSA 100 Mk 2 goniometer, Hamburg, Germany). We obtained the final results by averaging at least 10 separate runs.

RESULTS

Characterization of the Electrosprayed PLLA Microspheres

Figure 2 shows the morphology of the electrosprayed PLLA microspheres with the following electrospraying parameters: 6% solution concentration, 11-kV jet voltage, 15-cm collecting distance, and 0.02 mL/min flow rate. SEM images demonstrated that the electrospraying method could fabricate microspheres with uniform size [Figure 2(a, b)] and smooth surface [Figure 2(c)]. Part of the electrosprayed microspheres had a red-blood-cell-like concave morphology [Figure 2(c)]. The nanometer software statistically measured that the average size of the electrosprayed PLLA microspheres was 1.92 ± 0.35 μm. Figure 3

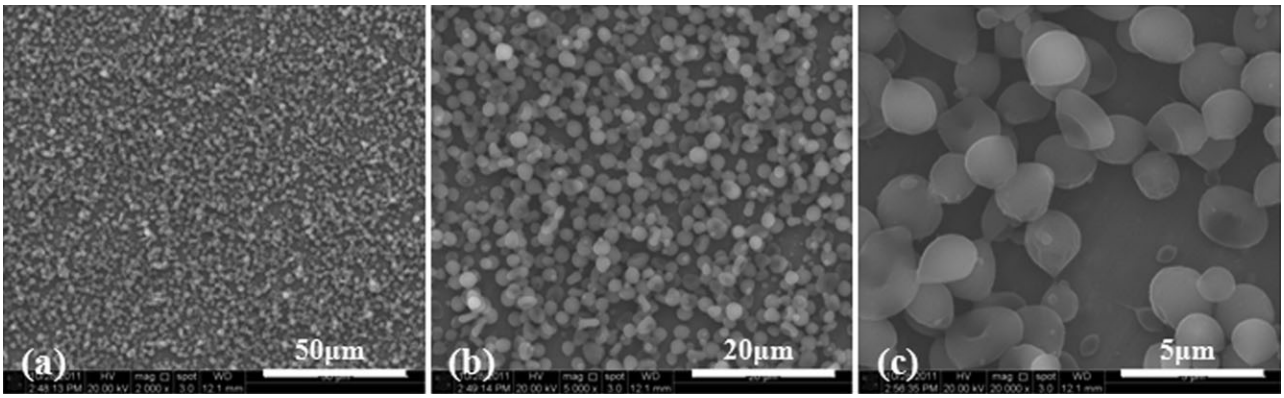

Figure 2. SEM morphologies of the electrosprayed PLLA microspheres. (The concentration of the electrospraying solution was 6 wt %, and the other electrospraying parameters were a voltage of 11 kV, a collecting distance of 15 cm, and a flow rate of 0.02 mL/min.)

demonstrates that the dynamic light scattering (DLS) method was used to measure the size and PDI of the electrosprayed PLLA microspheres, and the results show that the size of the electrosprayed PLLA microspheres was 2.01 ± 0.03 μm, with a PDI of 0.839; this indicated that the size of microspheres statistically had homogeneity. On the other hand, the size statistically measured from the SEM images by nanometer software was close to that shown in DLS measurement, which indicated the truth of these two measurements. The ζ potential of the electrosprayed PLLA microspheres was −4.55 ± 0.54 mV by a particle size analyzer; this demonstrated that the electrosprayed PLLA microspheres had a negative charge and that the electrosprayed PLLA microspheres were unstable in the PVA water.

Figure 4 shows the XPS spectra of the C element on the surface of the PLLA microspheres fabricated by the electrospraying method and the emulsion method. Table I demonstrates the peak assignments and the theoretical and experimental contents of each carbon environment. The numbers assigned to each peak were used to identify the presence of these carbon environments in the data analysis for the PLLA microspheres fabricated by the electrospraying method and the emulsion method.

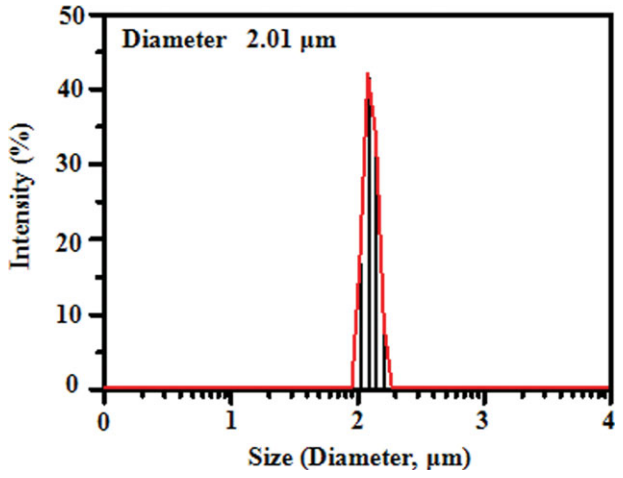

Figure 3. DLS measurement of the hydrodynamic diameter of the electrosprayed PLLA microspheres in a PVA solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

As shown in Figure 4 and Table Ithe contents of —CH_3 (284.5 eV), —CH⟨ (286.3 eV), and ⟩C= (288.4 eV) in the PLLA microspheres fabricated by the emulsion method were near each other; this was in accordance with the theoretical value. However, in the electrosprayed PLLA microspheres, the content of —CH_3 obviously increased from 33.3% for the microspheres prepared from the emulsion method to 46.4%, and the contents of —CH⟨ (286.3 eV) and ⟩C= (288.4 eV) decreased accordingly. The previous results indicate that the polymer groups on the surface of the PLLA microspheres rearranged during the high-voltage electrospraying process and that the hydrophobic —CH_3 enriched on the surface of the PLLA microspheres and hydrophilic ⟩C= distributed on the subsurface.

By the WCA measurement, the WCA of the electrosprayed PLLA microsphere surface was 142.0 ± 1.6° and that of the PLLA microspheres fabricated by the emulsion method was 128.6 ± 3.4°; this indicated that the membrane formed by the electrosprayed PLLA microspheres had strong hydrophobicity and that the single electrosprayed PLLA microspheres also had hydrophobicity.

Effect of the Electrospraying Solution Concentration on the Microspheres

The solution concentration is the main parameter influencing the electrosprayed PLLA microspheres. The change in the solution concentration led to a change in the surface tension and viscosity and, as a result, further influenced the size of particles. When the PLLA concentration changed from 3 to 6 wt %, the electrosprayed PLLA microspheres increased from 1.1 to 2.0 μm with increasing electrosprayed PLLA solution concentration (Figure 5); this was in accordance with the results of Xu et al.[27] and Weng et al.[28] At high solution concentration, polymer chain entanglement could easily occur; this led to the formation of microspheres with a large size.

The measured ζ potential was negative, and their moduli increased from 3.0 to 4.5 mV with increasing PLLA solution concentration. The previous results demonstrate that the size of the electrosprayed PLLA microspheres could be increased through an increase in the solution concentration with the decrease of the surface ζ potential value of the electrosprayed PLLA microspheres. The ζ potential indicates the colloidal stability of the

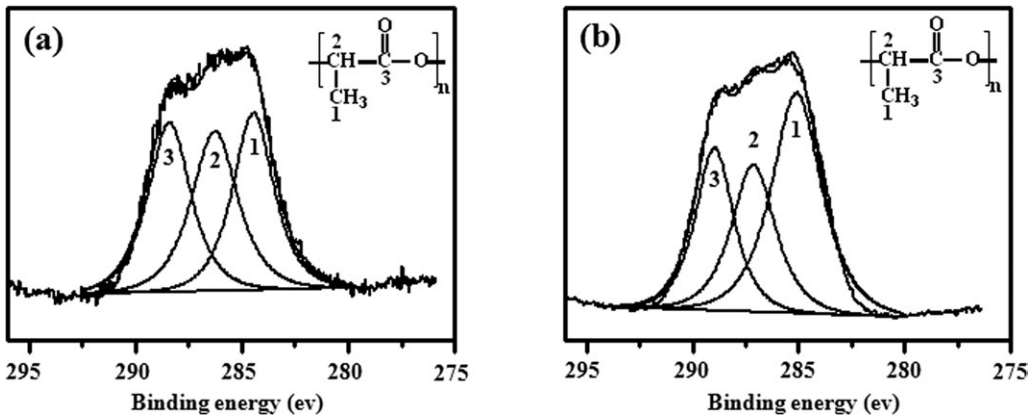

Figure 4. C1s regions and the peak assignments of the XPS signals for the PLLA microspheres fabricated by the (a) emulsion and (b) electrospraying methods.

microspheres, and the large absolute value of the ζ potential indicates better colloidal stability. As shown by the results of the ζ potential, the microsphere surface was negatively charged; this was attributed to the presence of ionized carboxyl groups from lactic acid in the water solution.[29] The size of the microspheres increased with increasing concentration; this led to an increasing number of ionized carboxyl groups on the surface. Thus, the ζ potential of the microspheres was more negative.

Effect of the Electrospraying Voltage on the Microspheres

The size of the electrosprayed PLLA microspheres decreased from 2.0 to 1.3 μm with increasing jet voltage (Figure 6); this was consistent with the scaling law of Hartman et al.[30] According to the scaling law

$$d = \alpha\left(\frac{\rho\varepsilon Q^4}{I^2}\right)^{\frac{1}{6}} \quad (1)$$

$$I \propto (\gamma K Q)^{\frac{1}{2}} \quad (2)$$

where d is the droplet diameter, α is a constant, Q is the liquid flow rate, ρ is the solution density, I is the current, ε is permittivity of a vacuum, γ is the surface tension, and K is the solution conductivity. We concluded from eqs. (1) and (2) that the diameters of the electrosprayed microspheres were inversely proportional to *I;* that is, the higher *I* was, the smaller the diameters of the electrosprayed microspheres were, and the lower *I* was, the bigger the diameters of the electrosprayed microspheres were. *I* was proportional to the jet voltage between the syringe and the collector; as a result, the higher jet voltage meant a smaller diameter for the electrosprayed microspheres. This was because when the jet voltage reached a certain value, the electrostatic repulsion overcame the surface tension and form jet; the coulomb breakup during the electrospraying process increased with increasing voltage and eventually resulted in a smaller diameter of the electrosprayed microspheres on the collector.

The ζ potential of the electrosprayed PLLA microspheres was negative, and their moduli decreased from 6.6 to 3.5 mV with increase voltage from 7 to 15 kV.

Table I. Experimental and Theoretical Contents of the C1s Regions of the XPS Signals for the PLLA Microspheres Fabricated by the Electrospraying and Emulsion Methods

Peak	Binding energy (eV)	Emulsion experimental content (%)	Microsphere experimental content (%)	Theoretical content (%)
1	284.5	33.3	46.4	33.3
2	286.3	33.7	27.4	33.3
3	288.4	33.0	26.2	33.3

Effect of the Electrospraying Flow Rate on the Microspheres

The size of the electrosprayed PLLA microspheres increased from 1.9 to 2.9 μm with increasing flow rate (Figure 7). This result conformed to Hartman's formulas [eqs. (1) and (2)], in which the diameters of the electrosprayed microspheres increased with increasing flow rate. When the flow rate increased, the liquid droplets sprayed out per minute increased accordingly; this made the charge density of the droplets decrease and led to fewer breakups and a large size of the particles on the collector.[5]

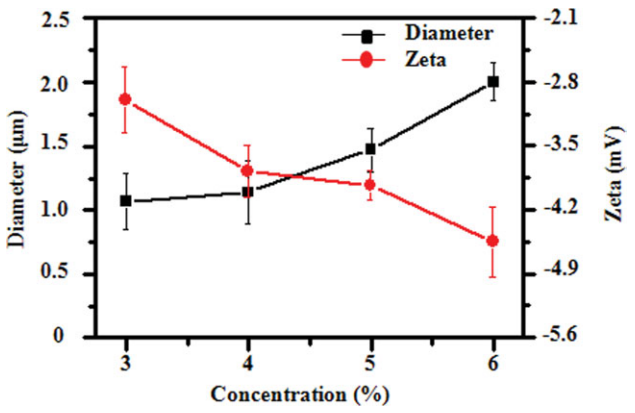

Figure 5. Effect of the solution concentration on the particle size and ζ potential of the electrosprayed PLLA microspheres (applied voltage = 11 kV, liquid flow rate = 0.02 mL/min, and collecting distance = 15 cm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

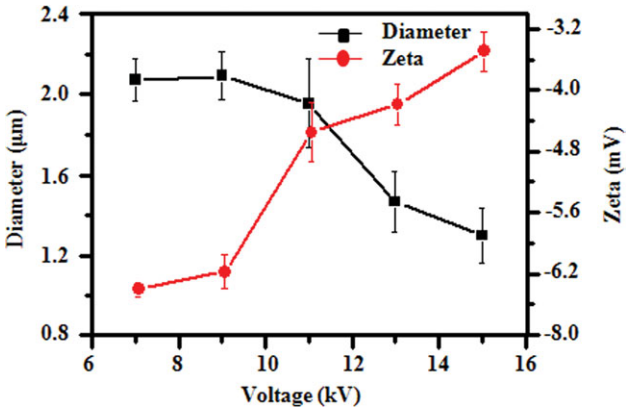

Figure 6. Effect of the voltage on the particle size and ζ potential of the electrosprayed PLLA microspheres (polymer concentration = 6 wt %, liquid flow rate = 0.02 mL/min, and collecting distance = 15 cm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

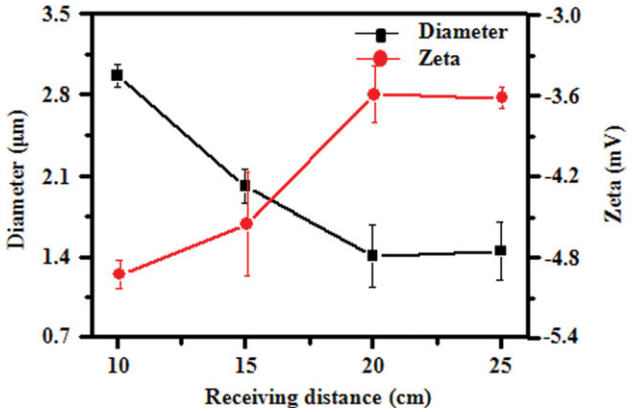

Figure 8. Effect of the collecting distance on the particle size and ζ potential of the electrosprayed PLLA microspheres (polymer concentration = 6 wt %, voltage = 11 kV, and liquid flow rate = 0.02 mL/min). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The ζ potential of the electrosprayed PLLA microspheres was negative, and their moduli increased from 4.6 to 6.9 mV with increasing flow rate from 0.02 to 0.10 mL/min.

Effect of the Collecting Distance on the Microspheres

The size of the electrosprayed PLLA microspheres decreased with increasing collecting distance from 10 to 20 cm. However, when the collecting distance was larger than 20 cm, the size of the electrosprayed PLLA microspheres basically remained unchanged (Figure 8). This phenomenon was related to the coulomb breakup during the electrospraying process. When the jet moved from the needle nozzle to the collector, the jet broke up into charged droplets, and the density of the charged droplets increased with the evaporation of the solvent. As a result, the density of the surface charge increased; this led to an increase in the electrostatic repulsion, caused more coulomb breakup, and resulted in smaller charged droplets. When the collecting distance was short, the solvent evaporation was not rapid enough to form a hard and dense shell on the surface of the charged droplets. Then, the coulomb breakup increased with increasing collecting distance; this led to the small size of the electrosprayed microspheres on the collector. When the collecting distance was long enough, there formed a hard and dense shell on the charged droplet surface and prevented further coulomb breakup. Then, the size of the electrosprayed microspheres increased with increasing collecting distance.

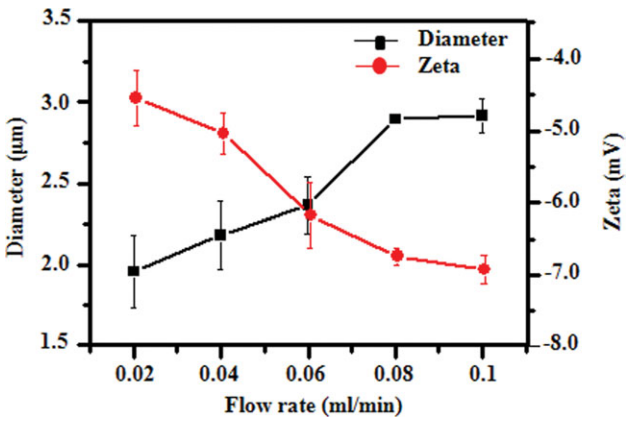

Figure 7. Effect of the flow rate on the particle size and ζ potential of the electrosprayed PLLA microspheres (polymer concentration = 6 wt %, voltage = 11 kV, and collecting distance = 15 cm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The ζ potential of the electrosprayed PLLA microspheres was negative, and their moduli decreased from 4.9 to 3.6 mV with increasing collecting distance from 10 to 25 cm.

DISCUSSION

The polymer solution was atomized under a high voltage of thousand or tens of thousands of volts into liquid sprays during the electrospraying process; this was a complex physical process. These liquid sprays had a high frequency of random motion under electrostatic force, broke up into smaller droplets with the rapid evaporation of the solvent, and finally dispersed on the collector as microspheres/nanospheres. The breakup of the sprayed droplets could be regulated by the control of the solution concentration, viscosity, and electrostatic force to generate polymeric microspheres/nanospheres on the collector. During the electrospraying process, the polymer chain transformed from the free state in the solution to the condensed state in the microspheres/nanospheres. The polymer groups with specific charge rearranged by electrostatic force and charge enrichment under high voltage, thus influencing the surface characterization of the electrosprayed microspheres/nanospheres. As described in this study, the enrichment of methyl groups on the surface of the electrosprayed PLLA microspheres contributed to the high hydrophobicity of the electrosprayed PLLA microsphere surface (as shown in Figure 1).

The fabrication of the microspheres by the electrospraying technique is a complex process in which the size and morphology of microspheres are influenced by the electrospraying parameters, such as the solution concentration, jet voltage, flow rate, and collecting distance. This study investigated the influence of

each parameter on the microsphere size and ζ potential. The results demonstrate that the size of the microspheres increased with increasing solution concentration and flow rate and decreased with increasing jet voltage and collecting distance. The results also indicate that the size range of the electrosprayed microspheres was 0.5–5.0 μm, and the microspheres within this size range easily circulated and metabolized in the body fluid; this makes the electrosprayed microspheres an ideal candidate for applications in drug-control-release carriers, such as in nasal drug delivery, spray drug delivery, and wound dressing. Thus, microspheres within a desired size can be fabricated by control of the parameters of the electrospraying technique.

The surface charge of microspheres is of interest because it influences the stability of a microsphere suspension and the interactions of the nanoparticles with cell membranes. In addition, from the ζ potential measurement, we can roughly determine the dominated component on the particle surface. Muller and coworkers[31–33] demonstrated that a high potential value of about −25 mV ensures a high-energy barrier that stabilizes the suspensions. From ζ-potential analysis, the PLLA microspheres prepared by the emulsion method had a negative charge of −32 mV on the surface. This was attributed to the presence of terminal —COOH groups in the polymers. However, the PLLA microspheres prepared by electrospraying exhibited ζ potentials between −3.0 and −10.0 mV, and a great decrease in the absolute value of the PLLA microspheres surface charge was observed. Because the PLLA microspheres were produced under a high voltage, the groups were rearranged, and the surface charge decrease demonstrated the presence of —CH_3 groups on the surface; this shifted the shear plane of the diffusive layer to a lower distance.[34] Therefore, a little surfactant could be adsorbed on the microsphere surfaces with the formation of a thinner surfactant film on the microsphere surface; this elicited a reduced electrophoretic mobility.[35] Thus, when the solution was used as a collector of the electrospraying process, the surfactant could be added to the collecting solution to prevent the agglomeration of the electrosprayed PLLA microspheres.

In the field of biology and medicine, the particle size (size and size distribution) and surface characterization (charge and hydrophobicity) of the microspheres influence a cell's adsorption and phagocytosis and determine the microspheres' cardiac–vascular cycle, circulate rate, target binding capacity, stability, and drug release rate. The sizes of microspheres fabricated by electrospraying were from 100 nm to 5 μm, and they could be used in injectable and respiratory drug delivery. The high hydrophobicity of the PLLA microspheres surface delayed the release time of the drug. Thus, this systematic research on the influence of electrospraying parameters on the size and surface characterization of electrosprayed polymeric microspheres provide a theoretical basis for preparing microspheres with desired size and surface characterization.

CONCLUSIONS

In this study, we investigated the influence of the electrospraying parameters (solution concentration, jet voltage, flow rate, and collecting distance) on the size and surface characterization of electrosprayed PLLA microspheres. The results demonstrate that the electrospraying technique was a one-step method for fabricating monodispersed PLLA spherical microspheres with sizes of 1.92 ± 0.35 μm and the enrichment of the methyl group on the surface of microspheres during the electrospraying process contributed to the strong hydrophobicity of the microspheres' surface. The size and ζ potential of the PLLA microspheres increased with increasing solution concentration and flow rate and decreased with increasing injection voltage and collecting distance. The results provide the theoretical basis for the investigation of the preparation of drug-loaded PLLA microspheres and controlled drug release.

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