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Tuning the Size of Poly(lactic-co-glycolic Acid) (PLGA) Nanoparticles Fabricated by Nanoprecipitation

*Wei Huang and Chenming Zhang**

Polymeric nanoparticles (PNPs) are promising drug carriers in cancer treatment. Size of the particles has a significant impact on drug loading, in vivo distribution, extravasation, intratumor diffusion and cell uptake, and thus is critical for the successful development of a drug delivery regime. However, methods for manufacturing PNPs of defined size are yet to be established. The goal of this study is to establish a method that can be used to fabricate PNPs with controlled size. The factors that could impact the size of PNPs fabricated by nano-precipitation are systematically investigated. The factors studied include polymer concentration, organic solvent, temperature, aqueous phase ionic strength, organic phase injection rate, aqueous phase agitation rate, gauge of the needles, and final polymer concentration. Polymer concentration, the choice of organic solvent, temperature, and the ionic strength of the aqueous phase are shown to have a significant impact on the size of PNPs, and the effect of these factors can be attributed to a single parameter, the diffusion coefficient of the solvent in water, D_{pw}. It is possible that by tightly control these four parameters, nanoparticles with highly predictable and desirable size with narrow size distribution can be fabricated.

1. Introduction

Polymeric nanoparticle (PNP) has showed much potential as a drug delivery vehicle. The size of PNPs is in the range of 10–1000 nm, and it provides PNPs with unique properties such as the ability to penetrate leaky blood vessels, to infiltrate into tissues, and to enter targeted cells. Besides the targeting abilities, other recent developments on drug loading and release have made nanoparticles in general very effective against many diseases such as cancer.[1] In mouse models, PNPs showed significant natural enrichment in solid tumors by the enhanced permeability and retention effect (EPR).[2] The surface of PNPs can be modified with ligands that allow them to precisely target the tumor microenvironment or organelles of tumor cells. For example, antibodies and small molecule drugs were delivered to the tumor cell surface to interrupt the cell growth or invasion.[3] Others reported to deliver chemotherapy and gene-therapy agents to the cytoplasm or nucleus through endocytosis[4] and lysosome escaping.[5] In another case, PNPs were used to deliver therapeutic agents to the brain tissue because PNPs can penetrate the blood-brain barrier (BBB) for the potential treatment of Alzheimer's disease or Parkinson's disease.[6] Other efforts have been made to treat organs[7,8] and infectious diseases.[9,10] Nevertheless, the transportation of PNPs from blood to tissues and into the pathological cells is strongly size dependent, and thus size of PNPs is a critical issue in all cases aforementioned.

In fact, in vivo studies of PNPs usually showed a rather broad distribution in different tissues. Instead of specifically accumulating at the tumor, large portions of PNPs are rapidly cleared from the blood circulation by the mononuclear phagocyte system (MPS) upon opsonization, or filtered out in other particular organs.[11] Studies showed this undesired clearance process is strongly affected by the particle size. Generally, PNPs larger than 200 nm are frequently sieved by sinusoid of spleen, 100–150 nm ones are captured by the Kupffer cells of liver, and the ones smaller than 5.5 nm are found to leak out from the kidney.[11] A study focused on investigating size dependent organ distributions of intravenously injected nanoparticles showed a distinguishable presence of 10 nm particles in blood, liver, spleen, kidney, testis, thymus, heart, lung, and brain. While the other larger particles (50, 100, and 250 nm) were only primarily found in blood, liver and spleen.[12] On the other hand, the comparative study on tissue distributions of nanoparticles around 200 nm with the ones over 1000 nm showed a higher tendency of MPS clearance for the larger particles.[13,14]

Cell uptake rates are also strongly affected by the size of PNPs. Cells internalize particles via different pathways depending on the size, generally, receptor-mediated endocytosis for particles of 100–200 nm and phagocytosis for the larger ones.[15–17] It was reported that 100 nm particles had the highest uptake rate by human epithelial colorectal adenocarcinoma cells (Caco-2) when 50, 100, 200, 500, and 1000 nm NPs were compared.[18] The rate decreased exponentially as the size exceeded 100 nm.[18] On the other hand, 150 nm NPs were found to have the optimal uptake rate by murine non-phagocytes.[19] The study of NP (ranging from 40–15 000 nm) uptake by dendritic cells showed similar results, as the particles smaller than 500 nm showed a favorable uptake rate.[20] On the other hand, uptake of NPs by human umbilical vain endothelia cells, ECV 304 and HNX 14C, favored microspheres up to 1010 nm, whereas Hepa 1-6 and HepG2 only up-took NPs smaller than 93 nm.[21]

The diffusion rate of NPs through the extracellular matrix (ECM) has to be considered when studying in tumor drug

Dr. W. Huang, Dr. C. Zhang
Biological Systems Engineering, Virginia Tech, Blacksburg, VA, USA
E-mail: chzhang2@vt.edu

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distribution,[22] and it was reported that only PNPs of 20–40 nm were found to penetrate into an enzyme treated tumor spheroid (i.e., the enzyme degrades ECM inside the tumor).[23] Larger PNPs, on the other hand, were found abundantly only at the surface.[22] The choice of the size of NPs for a targeted drug delivery is also affected by the drug loading capacity and the colloid stability. Larger particles commonly have a higher drug loading capacity, but the colloid stability was found to drop with the increase of the particle size above a threshold.[24] Hence, the size of the NPs needs to be precisely controlled to fulfill the intended use with the highest efficiency.

One efficient way of controlling the size of PNPs is by carefully selecting the fabrication technique and pertinent fabrication parameters.[25] Among the PNP fabrication methods (i.e., the bottom-up methods such as microemulsion polymerization, precipitation polymerization etc., and top-down methods such as emulsion diffusion, solvent displacement, etc.), the most widely used ones in producing drug nano-carriers include double emulsion,[26] nano-precipitation,[27] and spray drying.[28] The double emulsion and spray drying methods usually lead to the production of large PNPs (over 300 nm).[29,30] Nano-precipitation has been used to fabricate smaller PNPs (i.e., 100–200 nm). A rather complicated artificial neural network model was shown to predict the size of the tri-block polymer, poly(lactide)-ply(ethylene glycol)-poly(lactide), based PNPs.[31] There are some other recent developments on PNP fabrication that can allow fine control of the size of the particles. In the work by Kucuk et al., a microfluidic system was designed to manufacture PNPs of 80–920 nm controlled by the polymer concentration and feed flow rate.[32] Parhizkar et al. used an electrohydrodynamic atomization (EHDA) system to encapsulate drugs with poor solubility in organic solvents at high efficiency.[33] In other applications, the same group also used the EHDA system to fabricate nano-fibers by changing the properties of the feed and processing conditions.[34] Another technique to fabricate beads, beaded-fibers, and fibers is pressurized gyration.[35] Moreover, a study done by Contado et al. reported that the size of PLGA PNPs fabricated by nanoprecipitation was dependent on polymer concentration,[36] but many other factors that could potentially affect the particle sizes have not been studied. In this work, the focus is to fabricate small spherical PNPs by nano-precipitation. Poly(D,L-lactic-co-glycolic acid) (PLGA), a biodegradable material commonly used in drug delivery vehicles to minimize the carrier related side effects, was used to study the impact of the process on the particle sizes. The impacts of polymer concentration, type of organic solvent, temperature, aqueous phase ionic strength, organic phase injection rate, aqueous phase agitation rate, gauge of the needles, and final polymer concentration on the size of the PLGA PNPs were systematically studied. The results showed that the sizes of the fabricated NPs can be attributed to a single and easily determined parameter, the diffusion coefficient (D_{pw}) of the solvent in water with the presence of the polymer.

2. Experimental Section

2.1. Materials

PLGA (50:50, molecular weight (MW): 30 000-60 000) and polyvinyl alcohol (PVA, MW: 50 000) were purchased from Sigma–Aldrich (St. Louis, MO). Solvent and other chemicals were purchased from Fisher Scientific (Pittsburg, PA) unless otherwise specified. Deuterium oxide was purchased from Cambridge Isotope Laboratories (Andover, MA).

2.2. PLGA PNP Fabrication by Nano-Precipitation

Predetermined amount of PLGA was dissolved in the organic phase of selected solvent and injected at a predetermined rate into the aqueous phase by a vertically mounted syringe pump with magnetic stir agitation. The resulted suspension was placed under vacuum overnight with agitation in a safety fume hood to eliminate the organic solvent. Powder PNPs were produced from the liquid suspensions by freeze drying.

2.3. Size and Zeta Potential Measurement

Size distribution and zeta potential of PLGA PNPs were analyzed on a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA). Samples were freshly prepared before use by adding aliquots of PNPs to 0.01 M NaCl buffer to make a solution with a PLGA concentration of 0.1 mg mL^{-1}. During the test, samples were injected into a disposable capillary cell DTS1060 (Malvern Instruments, MA) and loaded onto the analyzer. Measurements were taken at 25 °C with a material refraction index of 1.33, and viscosity of 0.8872 cp.

2.4. Morphology Study of PLGA PNPs

Transmission electronic microscopy (TEM) images were taken for morphology study. Briefly, liquid samples were deposited onto carbon coated copper grids for 5 min. Phosphotungstic acid of 2% was used for negative staining for 30 s. TEM images were taken by a JEOL JEM 1400 (JEOL Ltd, Tokyo, Japan).

2.5. Study of Factors That May Affect the PNP Size

Impacts of polymer concentration, organic solvent, ionic strength of aqueous phase and temperature of aqueous phase on particle size were studied. To make the PNPs, 2 mL of the organic phase (10 mg mL^{-1} PLGA acetonitrile solution) was injected into 100 mL filtered 0.1% PVA aqueous solution at a rate of 2 mL min^{-1} at 25 °C for each case unless otherwise specified.

For the polymer concentration test, final concentrations of 1, 5, 10, 20, and 40 mg mL^{-1} PLGA in acetonitrile were made with brief sonication. For the solvent test, the organic phase was made by dissolving PLGA in three types of solvent, acetonitrile, acetone and tetrahydrofuran (THF), with a final concentration of 10 mg mL^{-1}. For the ionic strength test, the organic phase was injected into an aqueous phase containing increasing amount of sodium chloride (0, 0.1, 1, 10, 100, and 1000 mM). For the temperature test, the aqueous phase was adjusted to the predetermined temperature (0, 10, 20, 30, 40, 50, 60, 70, and 80 °C) before injection of the organic phase.

Impacts of organic phase injection rate, aqueous phase flow rate, gauge of the needle and final concentration of polymer in the suspension on particle size were studied. Again, to make the

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For the injection rate test, four flow rates of the organic phase were compared, 2, 20, 200, and 2000 μL min^{-1}. For the aqueous phase flow rate test, decreasing magnetic stir agitation speed was applied to the aqueous phase (1200, 1100, 700, 350, 125, and 0 rpm). For the needle gauge test, five commonly accessible syringe needles were used for the injection (14G, 16G, 20G, 23G, 27G). For the final polymer concentration test, predetermined volume of organic phase was constantly added to 2 mL of aqueous phase to make the final PLGA concentration in water from 0.1 to 10 mg mL^{-1}. The increase of particle number resulted from higher PLGA concentrations was monitored by flow cytometer (FCM).

2.6. Diffusion Coefficient Measurement

All nuclear magnetic resonance (NMR) diffusion experiments were performed with a Bruker Avance III NB 600 MHz (14.1 T) NMR spectrometer, using a TBI single-axis gradient probe and a 5 mm inner coil. The diffusion measurements were acquired with ^{1}H signal with pulsed-gradient stimulated-echo pulse sequence.

3. Results

3.1. Influence of Polymer Concentration in the Organic Phase on Particle Size

In order to test the influence of the polymer concentration in organic phase on the particle size, we fabricated PLGA PNPs with 1 to 40 mg mL^{-1} PLGA acetonitrile solution. The size distribution of the resulted PNPs were measured and shown in **Figure 1**A. In the range of 0–20 mg mL^{-1}, the PLGA particles showed a steady increase in size as more concentrated PLGA was used in the preparation. At those concentrations, monodispersed PLGA PNPs were fabricated as indicated by the narrow distribution of size. However, no further increase in size was observed for the PNPs fabricated using a 40 mg mL^{-1} solution. Instead, majority of the PNPs had the same size distribution as when a 20 mg mL^{-1} solution was used, and the formation of large particles (over 500 nm) was observed at this high concentration of polymer in organic phase. Thus, a PLGA concentration of lower than 20 mg mL^{-1} could be used to control the size of the PNP. The dependence of the mean PNP size on the polymer concentration is shown in Figure 1B. Evidently, in the 0–20 mg mL^{-1} range, the PNP size had a linear correlation with polymer concentration. Since the PNPs were formed *via* precipitation of the polymer from the organic droplets in the aqueous phase,[37] the size of the PNP could possibly be, at least in part, predicted by the diffusion rate of the organic phase in the aqueous phase. The impact of diffusion rate of the organic solvent on the size of PNPs was therefore carefully monitored in the rest of this study.

3.2. Influence of the Organic Solvent on Particle Size

Two important properties of the organic solvent used for nanoprecipitation are the polymer solubility and water miscibility.[27] The most commonly used solvents include acetonitrile, acetone, ethanol, and methanol. A solvent is usually selected empirically. The effect of solvent on the resulted PNP size is still not clear.[25] Thus, the commonly used organic solvents were tested. Representative size distribution and mean particle size results are shown in Figure 1C and D, respectively (data for ethanol and methanol are not shown due to the low solubility of PLGA in the two solvents). As it can be seen that the size of the PNPs varies in the order of acetonitrile < acetone < THF. To further understand the effect of different organic solvent on particle size, the diffusion coefficient (D_{pw}) of the solvent with/without the presence of PLGA in water was measured by NMR. The results show a trend of acetonitrile > acetone > THF in both cases (**Table 1**). It is hypothesized that the D_{pw} of the solvent in water with the presence of the polymer can be a good indicator of the corresponding PNP size and distribution. Solvents with high D_{pw} favor the formation of smaller PNPs with narrower distribution, meanwhile solvents with low D_{pw} result in large and broad distributions of size. The other solvents (data not shown) tested also support the hypothesis. Methanol and ethanol have a high D_{pw} and resulted in small size distribution within the 50–100 nm range. However, the solubility of PLGA in those solvents is relatively low compared to that in acetonitrile. On the other hand, no formation of PNP was observed using dimethyl sulfoxide or DCM. Instead, the solvent polymer mixture formed an oily layer at the bottom of the reactor. With agitation, PNPs with an extremely broad size distribution (i.e., microspheres) were formed after the removal of the organic solvent.

3.3. Influence of Temperature on Particle Size

The viscosity of water changes slightly with temperature. Conceivably, changes of the temperature could shift the D_{pw} of the solvent and therefore provide a fine adjustment of the PNP size. The temperature dependence of size distribution and average size are shown in **Figure 2**A and B, respectively. Indeed, there is an approximate 10 nm decrease of particle size with every 10 degree increase of temperature (Figure 2A). Overall, the mean diameter of the particles decreased for about 100 nm when the temperature was increased from 0 to 80 °C (Figure 2B). This could also in part be explained by the increased solubility of PLGA at higher temperatures, which inhibits precipitation. On the other hand, including PLGA, some polymeric materials undergo fast hydrolytic degradation especially at high temperature.[38] Similarly, degradation or denaturing of the loaded biomaterials (i.e., protein, nucleic acids) may also occur. However, it is worth to mention that the precipitation duration is relatively short compared to the degradation of the polymer and potential contents the particles may carry. It was observed that the NPs precipitated instantly during the solvent mixing. Temperature changes immediately after the precipitation (in case it is necessary to cool down the solution) had no effect on the particle size (data not shown).

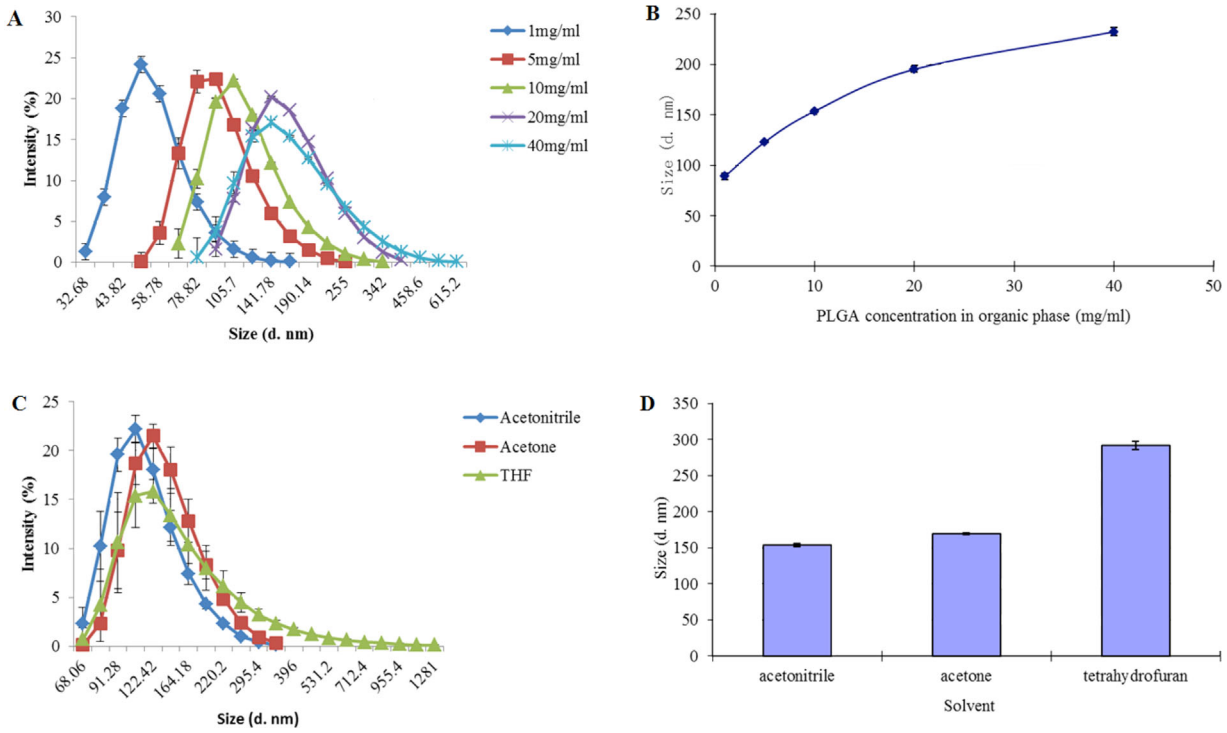

Figure 1. Particle size and distribution depend on PLGA concentration in organic phase and organic solvent used. A) Influence of PLGA concentration on particle size distribution, (B) mean particle size versus PLGA concentration, (C) influence of the choice of organic solvent on particle size distribution, and (D) mean particle size versus organic solvent used. A total of 2 mL of PLGA in acetonitrile was injected into 100 mL of 0.1% PVA aqueous solution at a rate of 2 mL min^{-1} at 25 °C. The mean size is the weighted average size of the population. (mean ± s.d., $n = 3$).

3.4. Influence of the Salt Concentration on Particle Size

The inclusion of salt in macroionic suspensions is known to cause the formation of a highly ordered crystal-like network of the charged colloid particles with the counter ions and thus increase the viscosity of the system.[39] Therefore, the salt concentration could have an impact on the diffusion coefficient of the solvent and thus the PNP size. A number of NaCl solutions (0 to 1M) were tested. As expected, a strong dependence of the size of PNPs on the salt concentration was observed (**Figure 3**A). Moreover, 10 mM of NaCl seems to be a critical concentration where a significantly broadened size distribution was observed and possibly resulted from the high viscosity of the solution. The trend of diffusion coefficient of the PNPs with the increase of NaCl concentration are shown in Figure 3B. The 10 mM data point shows a significantly larger error than those under other salt concentrations, which is most likely due to the high viscosity and D_{pw} variation in corresponding to small concentration changes around 10 mM. The high sensitivity of viscosity and D_{pw} indicate the 10 mM region is a very significant range for the transformation from PNP free dispersion to the ordered crystal-like network to occur, as also evidenced by many other works using poly(2-vinyl pyridine), sodium polystyrene sulfonate and poly-L-lysine.[38] The mean particle size as a function of the salt concentration is shown in Figure 3C. Evidently, the mean size of particles under NaCl concentrations of 100 and 1000 mM exceeds 1000 nm (microspheres), which are out of the range of the preferred PNPs for intravenously tumor drug delivery.

Table 1. Diffusion coefficient of organic solvent with/without the presence of PLGA in water (D_2O).

Organic solvents		Solvent diffusion coefficient, $m^2 s^{-1}$
With PLGA in water	Acetonitrile (10%)	1.538E-9
	Acetonitrile (10%)	1.217E-9
	THF (10%)	1.040E-9
Without PLGA in water	Acetonitrile (10%)	1.534E-9
	Acetonitrile (10%)	1.152E-9
	THF (10%)	1.000E-9

Samples were prepared as follow: 10 mg mL^{-1} PLGA in an organic solvent was first prepared, and 0.1 mL of the PLGA-solvent solution was mixed with 0.9 mL of D_2O. For the samples without PLGA, 0.1 mL of an organic solvent was mixed directly with 0.9 mL of D_2O.

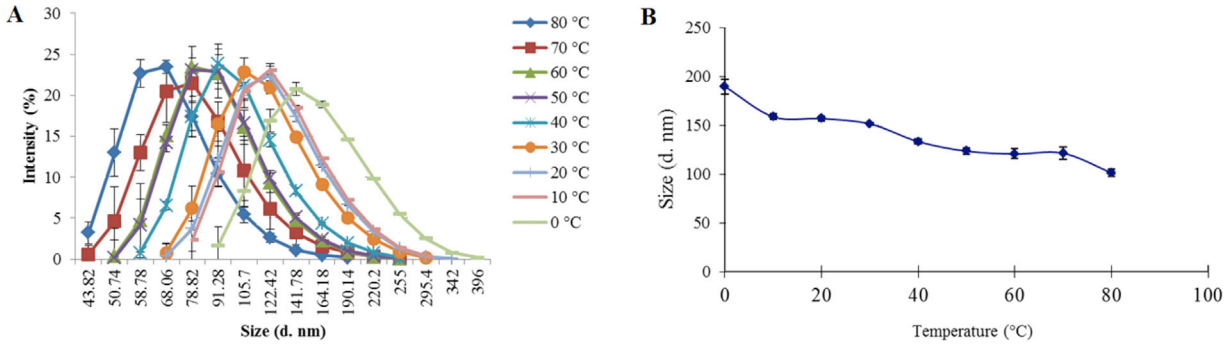

Figure 2. Temperature affects the size of PLGA nanoparticles. A) Influence of aqueous phase temperature on particle size distribution, and (B) mean particle size versus temperature. A total of 2 mL of 10 mg mL^{-1} PLGA in acetonitrile was injected into 100 mL of 0.1% PVA aqueous solution at a rate of 2 mL min^{-1} (mean ± s.d., $n = 3$).

3.5. Other Factors That May Affect Particle Size

Influences of organic phase injection rate, aqueous phase agitation rate and gauge of the needles on the size of PNPs were also investigated. The three parameters showed no significant effect on the PNP size (data not shown). On the other hand, since the three parameters only affect the rate of mass transport but not D_{pw}, as expected, the size of the PNPs were found unchanged even when extreme values such as 2 mL min^{-1} (for organic phase injection rate) and 0 rpm (for aqueous phase agitation speed) were used. These results further validate the hypothesis that the diffusion coefficient of the solvent plays the most critical role in the size of the fabricated PNPs.

3.6. Influence of the Final Polymer Concentration on Particle Size

To determine the endpoint of the nanoparticle fabrication process, the size of PNPs precipitated with increasing final concentrations were evaluated. The mean size remained steady within the 0–1 mg mL^{-1} range and slightly increased within 1–10 mg mL^{-1}

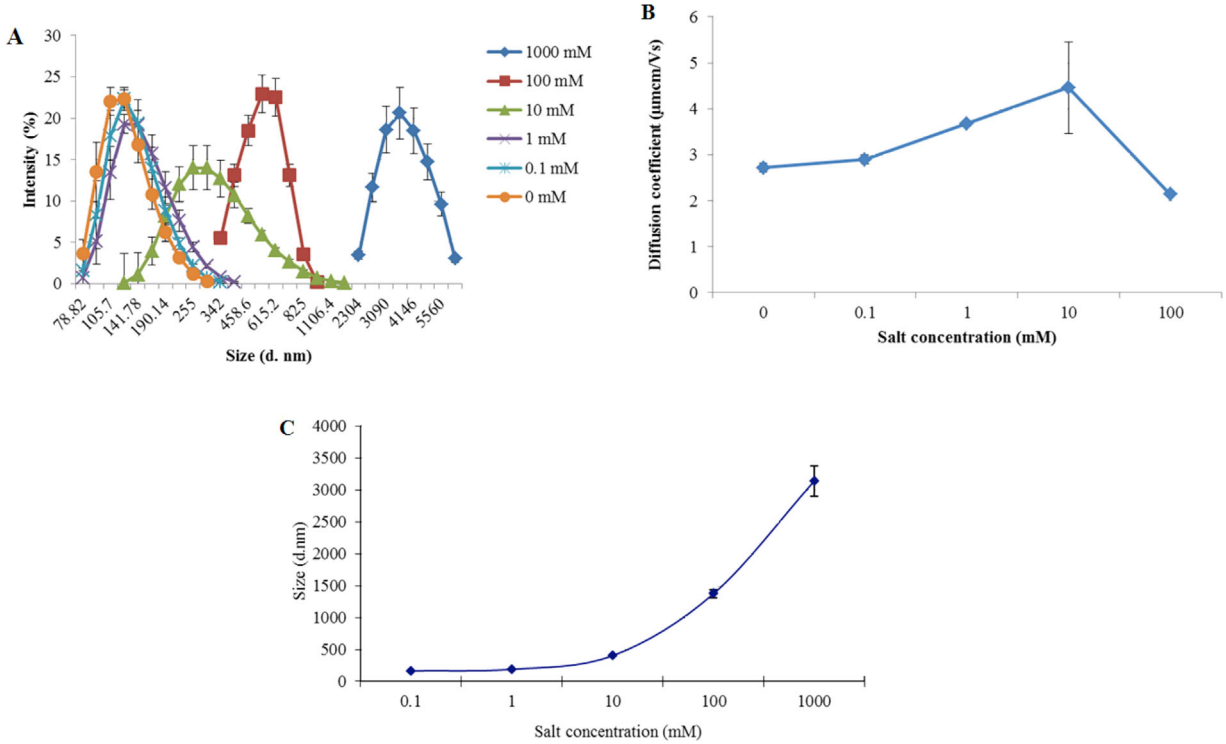

Figure 3. Salt (NaCl) concentration affects the size of PLGA nanoparticles. A) Size vs. salt concentration, (B) diffusion coefficient of PNP vs. salt concentration, and (C) Mean size vs. salt concentration. A total of 2 mL of 10 mg mL^{-1} PLGA in acetonitrile was injected into 100 mL of 0.1% PVA aqueous solution with different NaCl at a rate of 2 mL min^{-1} at 25 °C. (mean ± s.d., $n = 3$).

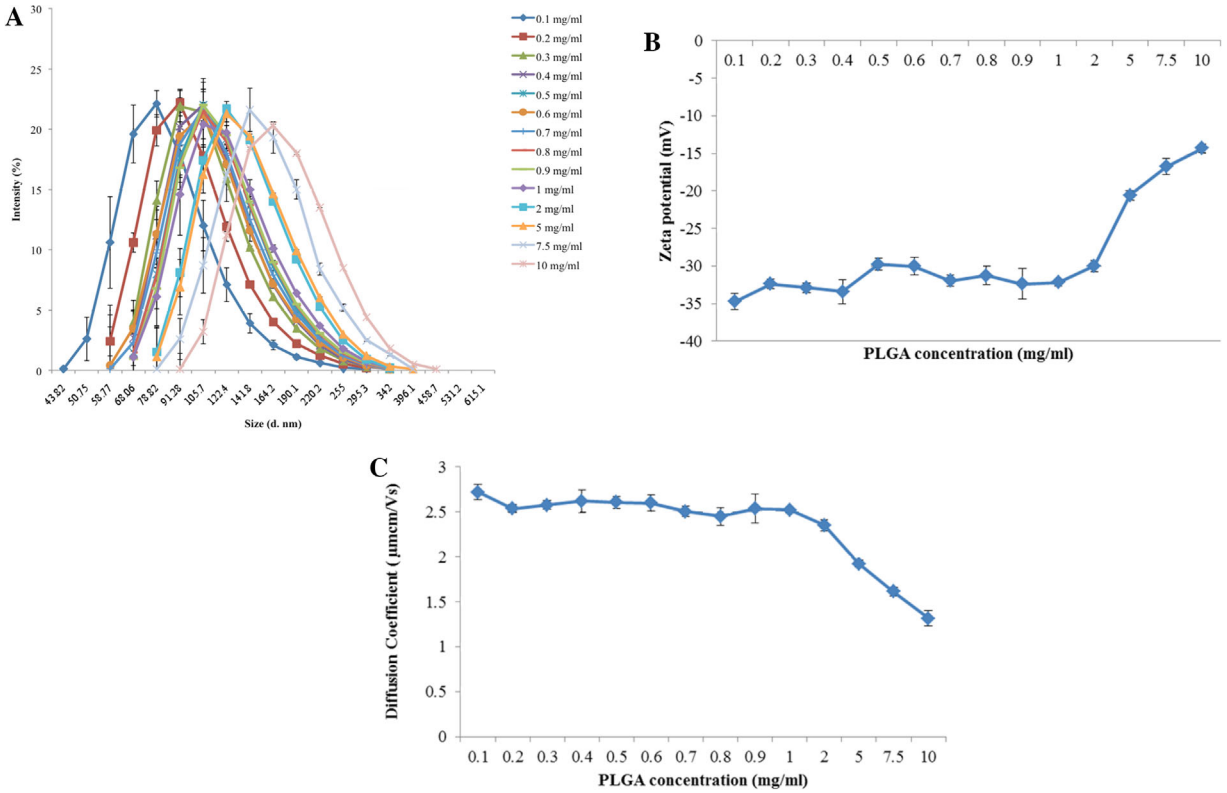

Figure 4. Final concentration of PLGA affects the size of PLGA nanoparticles. A) Size versus final concentration, (B) zeta potential versus final concentration, and (C) diffusion coefficient of PNP versus final concentration. Certain amount of 10 mg mL^{-1} PLGA in acetonitrile was injected into 100 mL of 0.1% PVA aqueous solution at a rate of 2 mL min^{-1} at 25 °C (mean ± s.d., $n = 3$)

(data not shown). Size distributions are shown in **Figure 4**A. Although the mechanism of how the concentration of polymers in the aqueous solution (in the form of macroions) affects the viscosity is still unclear, a few theories can help explain the data presented: (1) within the low concentration range (lower than a few mg mL^{-1}), the viscosity of the suspension will increase with the concentration but reach its maximum value and then remain stable thereafter; (2) above a certain threshold (depending on polymer type), the high concentration will lead to less charged particles due to the compressed electric double layer and increase of the viscosity.[40] In agreement with the theory, from 0.1 to 10 mg mL^{-1}, the resulted size distribution showed a slow increase to steady then to a fast increase trend (Figure 4A), indicating 0.3 to 1 mg mL^{-1} is the viscosity steady region (Figure 4C). The particle number, monitored by FCM, was found to increase linearly with the final PLGA concentration within this region, and this explains the relatively unchanged mean size when the PLGA concentration increases in the final aqueous solution. Further increase in PLGA concentration could result in increased viscosity and D_{pw} and therefore lead to the precipitation of larger particles. Noticing a mode size of 170 nm was observed for the PLGA concentration as high as 10 mg mL^{-1}, indicating the wide coverage of concentration by nano-precipitation to get PNPs within the desirable size range (50–200 nm) for in vivo drug delivery use. To further test whether the increase of particle size when PLGA concentration was above 1 mg mL^{-1} was due to the reduced charge density at PNP surface and the related viscosity increase, trends of zeta potential of the PNPs and diffusion coefficient of the solvent were monitored. Results are shown in Figure 4B and C, respectively. The increase in zeta potential within the 1–10 mg mL^{-1} region indicates the neutralizing process of PNPs when space becomes limited and the delocalization of carboxyl protons are restricted. The decrease of charge density of PNPs resulted in an increase of the viscosity of the system, as indicated by the diffusion coefficient data (Figure 4C). Spontaneous agglomerations could occur under such conditions and lead to reduced shelf life of the PNPs. Therefore, fabrication and storage of PLGA PNPs above the concentration of 1 mg mL^{-1} should be avoided.

4. Discussion and Conclusion

PNPs can serve as a versatile drug delivery vehicle. Their targeting functions are, however, largely affected by the size. In the case of a tumor targeting and drug delivery, currently a commonly recognized size range for the PNPs is 100–200 nm. Variations could have negative impacts on the in vivo distribution, in tumor spreading and the cell uptake rate, and therefore cause a loss of drug potency and side effects. The PNP size could be controlled by tuning the synthesis method and parameters of operation. Some research showed the loading of drug and surface modifications can cause swelling of the particles, but the increases in size are minor and predictable. Therefore, accurate control of the PNP size during the fabrication process is critical.

In this study, the focus was to control the size of PLGA PNPs fabricated by nano-precipitation, a process known to fabricate PNPs suitable for cancer drug delivery (100–200 nm, spherical). Impacts of several parameters on the particle size were tested, including the concentration of polymer in the organic phase (1–40 mg mL^{-1}), organic solvent (acetonitrile, acetone, THF), temperature (0–80 °C), ionic strength of the aqueous phase (0.1–1000 mM NaCl), flow rate of injection of the organic phase (2–2000 μL min^{-1}), agitation rate (0–1200 rpm), gauge of the needle for injection (14-27G), and final concentration of polymer (0.1–10 mg mL^{-1}). The first four were shown to have a significant impact on the PNP size. Among them, ionic strength, polymer concentration in the organic phase, temperature, and solvent could be used in combination for accurate size control. The last four parameters showed no significant impact on the PNP size. However they are beneficial from the manufacture point of view. For example, a reactor with multiple injection points and continuous agitation will be suitable for high throughput production of PNPs without affecting their size.

We hypothesize that the resulting size can be predicted by the diffusion coefficient of solvent in water with the presence of the polymer, D_{pw}. D_{pw} is determined by the solvent, polymer physicochemical properties and the system viscosity. Large D_{pw} results in narrowly distributed and smaller PNPs, while small D_{pw} can cause increase of overall size and distribution broadening. The dependence of PLGA PNP size on parameters such as the choice of organic solvent, operation temperature, and salt concentration was shown to attribute to D_{pw}. On the other hand, the particle size was unaffected by parameters that were not relevant to D_{pw}, such as feed rate, agitation rate. For instance, from 0.3 to 1 mg mL^{-1} PLGA PNP in water, the D_{pw} of the acetonitrile was relatively stable, leading to an unchanged size distribution of the PLGA PNPs in this range. For concentrations outside of this range, the particle sizes evidently was affected by the change of D_{pw}.

Although this study focused on the fabrication of small PNPs for drug delivery, PNPs of larger size and other shape are of interest in the field. Formation of large PNPs was observed in cases of (1) high concentration of polymer in the organic solution; (2) organic solvent of very low D_{pw}; and (3) high salt concentration. Some of these led to a polydispersed PNPs. Those parameters should be avoided, or the product would require further purification. Adding salt to the aqueous solution introduces rapid changes in the size of the PNPs in a large range (100–5000 nm). While, temperature allows small tunings of the size (10 nm/10°). It is very promising to use those two parameters in combination. However, the use of either is limited by the stability of materials under the selected conditions. In summary, multiple processing factors were shown to significantly affect the size of PNPs. By selecting appropriate factors, it is possible to fabricate PNPs with desired size.

Abbreviations

PLGA, poly(lactic co-glycolic acid); PNPs, polymeric nanoparticles; PVA, polyvinyl alcohol; TEM, transmission electronic microscopy; THF, tetrahydrofuran.

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Keywords

polymeric nanoparticles, nano-precipitation, particle size, PLGA, bioprocess

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