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Original Article

Microfluidic-assisted preparation of PLGA nanoparticles for drug delivery purposes: experimental study and computational fluid dynamic simulation

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Abstract

This study, for the first time, tries to provide a simultaneous experimental and computational fluid dynamic (CFD) simulation investigation for production of uniform, reproducible, and stable polylactic-co-glycolic acid (PLGA) nanoparticles. CFD simulation was carried out to observe fluid flow behavior and micromixing in microfluidic system and improve our understanding about the governing fluid profile. The major objective of such effort was to provide a carrier for controlled and sustained release profile of different drugs. Different experimental parameters were optimized to obtain PLGA nanoparticles with proper size and minimized polydispersity index. The particle size, polydispersity, morphology, and stability of nanoparticles were compared. Microfluidic system provided a platform to control over the characteristics of nanoparticles. Using microfluidic system, the obtained particles were more uniform and harmonious in size, more stable, monodisperse and spherical, while particles produced by batch method were non-spherical and polydisperse. The best size and polydispersity index in the microfluidic method was obtained using 2% PLGA and 0.0625% (w/v) polyvinyl alcohol (PVA) solutions, and the flow rate ratio of 10:0.6 for PVA and PLGA solutions. CFD simulation demonstrated the high mixing intensity of about 0.99 at optimum condition in the microfluidic system, which is the possible reason for advantageous performance of this system. Altogether, the results of microfluidic-assisted method were found to be more reproducible, predictable, and controllable than batch method for producing a nanoformulation for delivery of drugs.

Keywords: Computational fluid dynamic; Microfluidics; Nanoparticles; Nanoprecipitation; Polylactic-co-glycolicacid.

INTRODUCTION

In the recent years, due to the rapid development of the throughput for drug synthesis and analysis, a myriad of investigations have been carried out on developing novel and efficient therapeutic compounds. Among these compounds, some cases are insoluble, complex, unstable, and have high molecular weight. It is crucial to carry out an authentic delivery system for these drugs to ensure they achieve the target site and keep adequate treatment.

Nanoparticles have the ability to dissolve and entrap drugs and have been discovered as desirable drug delivery systems due to their smaller particle size, controlled, and enhanced drug release (1,2). They can be used for various routes of administration including oral, nasal, parenteral, intra-ocular, etc.(3). The higher intracellular uptake (4), deep penetration into tissues (5,6), controlled drug distribution to the target organ, and reduced drug exposure (7) are some of the advantages of nanoparticles.

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Two essential characteristics of nanoparticulated systems are particle size and size distribution, which are correlated with targeting capability, cytocompatibility, stability, loading and release of drug.

Nanoprecipitation is a one-step method involving straightforward and gentle formulation without requirement of any chemical additives or harsh formulation processes (8). In this approach, polymer dissolves in a water miscible organic solvent and adds to an aqueous solution (non-solvent). Based on the difference in solubility of polymer in organic solvent and aqueous solution, polymer nanoparticles will be produced spontaneously, and organic solvent diffuses in aqueous solution. This technique needs efficient mixing of polymer and aqueous solutions to fabricate homogenous nanoparticles (9). There are also other techniques available for fabrication of polymeric nanoparticles, but poor stability, limited control on particle size and morphology in these methods cause limitation in their application compared to nanoprecipitation technique (10).

In the recent years, microfluidic systems have attracted a lot of attention and have grown up as a new technology to generate micro/nano-sized particles (11-13). Microfluidic devices can manipulate and transport comparatively small volumes of fluids within a miniaturized area. The high ratio of surface area to volume in these devices causes efficient mixing and excellent mass transfer. Using microfluidic systems for generating nanoparticles is very advantageous due to better control over the experimental parameters and particle size (14), increasing the accuracy and efficiency of synthesis, adaptability for various multi-step processes, generation of homogenous particles, the online modification of nanoparticles without stopping the process (6), reduction in material consumption and operation costs, and increasing process safety.

Due to these conditions, a microfluidic-based system has been performed in this study to generate PLGA nanoparticles using nanoprecipitation method. Microfluidic systems result in formation of uniform nanoparticles with tunable size and polydispersity, due to the rapid and tunable mixing in a narrow junction area. In microchannels, two liquid streams come into contact at the crossing of channels. The governing flow regime in microfluidic devices is laminar; as a result, the dominant mass transfer phenomenon between two streams is diffusion within the confluence area. In microfluidic-assisted nanoprecipitation, the particles can be fabricated by three main steps: super saturation of solution (because of solvent change), the formation of nuclei (nucleation), and finally growth of the formed nuclei (15). A diffusion process generates local supersaturation, and the turbulences at the interface of streams (diffusion layer) result in efficient mixing, drive nucleation and crystal growth (16-18). It is regarded that a higher level of super saturation leads to increasing the nucleation rate compared to growth rate and causes fabrication of smaller size crystals (12).

The principal objective of this research is fabrication of PLGA nanoparticles by microfluidic-assisted nanoprecipitation and examination of the effect of concentration of surfactant and flow rates ratio of aqueous to organic phases on the morphology, size, and polydispersity of nanoparticles. Also, computational fluid dynamic (CFD) simulation was used to specify how fluids move and mix. CFD simulation gives complete information about mass transfer and fluid hydrodynamic in microfluidic systems using Navier-Stokes equations (19).

MATERIALS AND METHODS

Materials

PLGA (PLGA 50:50, 45 kDa) was purchased from Purac, Gorinchem, Netherlands. Dimethylsulfoxide (DMSO) and dioxane were procured from Merck, Germany. Polyvinyl alcohol (PVA; 13-23 kDa, 98% hydrolysis) was supplied by Sigma, USA.

A plus shape flow-focusing microfluidic chip was fabricated on a teflon flat plate by precise milling (teflon was chosen because of its solvent resistance). The microchip contains three inlet branches with 800 μm in diameter, 15 mm in length, and a circular cross-section. Solutions were injected

into the chip by two syringe pumps (SP1000, FNM Co., I.R. Iran). In order to achieve efficient mixing and increase the confluence area, PVA solutions were injected symmetrically from two sides of the PLGA inlet channel.

Experimental procedure

The applied mechanism of nanoparticle preparation in this study is nanoprecipitation using microfluidic chip. PLGA was dissolved in DMSO with concentration of 2 % (w/v), and PVA was dissolved in distilled water with concentrations of 0, 0.0625, 0.125, 0.25, 0.5, 1, 1.5 and 2% (w/v). After injecting PLGA and PVA solutions to the microchip, DMSO started to diffuse into the aqueous phase due to its high miscibility in water, and PLGA nanoparticles precipitated out.

The produced nanoparticles were separated from dispersion medium by ultracentrifuge (70Ti, Beck man Co., USA) for 20 min at 23,000 rpm and 4 °C to remove PVA. The schematic presentation of the process is shown in Fig. 1.

To obtain the optimum flow rates, 2% (w/v) PLGA solution and 1% (w/v) PVA solution were injected to the chip with different flow rate ratios of PVA:PLGA (10:1.5, 10:1, 10:0.8, 10:0.6, 10:0.4, and 10:0.3). Prepared samples were analyzed by a Zetasizer (Nano-ZS, Malvern, UK) for the size and polydispersity index (PDI), and finally, the best ratio of aqueous to organic phase was chosen. After achieving the optimum flow rates ratio, all the experiments were carried out at the optimum flow rates.

In order to study the effect of surfactant concentration on particle size and PDI, PLGA solution (2%) and PVA solutions (0, 0.0625, 0.125, 0.25, 0.5, 1, 1.5 and 2%) were fed into the chip channels by two syringe pumps with the rates of 10 to 0.6 mL/h for PVA and PLGA solutions, respectively.

The batch precipitation was performed as a reference; the concentrations of polymer solution were similar to those in a continuous process at optimum conditions (PVA 1% and PLGA 2%). Using a sampler (Transferpette, Germany), 0.6 mL of PLGA solution was added dropwise to 10 mL of under-stirring solution of PVA for 1 min. Then, stirring the mixture continued for further 30 min to ensure that PLGA nanoparticles have been successfully precipitated out. The resulted solution was centrifuged for 20 min at 23000 rpm and 4 °C.

Nanoparticle characterization

Particle size analysis

Prepared nanoparticles were analyzed using a Zetasizer for their size and PDI. All particle size measurements were performed in phosphate buffered saline (PBS, pH 7.4) using a He-Ne laser beam at 658 nm with a scattering angle of 173°.

PDI was also measured using Zetasizer. PDI is an essential criterion that indicates the size distribution and similarity between particles in nanosuspension technology. The extensive PDI means a very broad distribution that causes irregular pharmacokinetic parameters and influences the therapeutic efficiency of a drug formulation. A PDI value of around 0.1-0.2 is desirable and points out a narrow size distribution (20).

Atomic force microscopy

The atomic force microscopy (AFM, Nanosurf® Mobile S., Switzerland) in the non-contact mode was used to image the shape and size of nanoparticles prepared by the microfluidic and batch methods. A drop of diluted aqueous dispersion was placed on a washed mica slide and dried under atmosphere for 24 h.

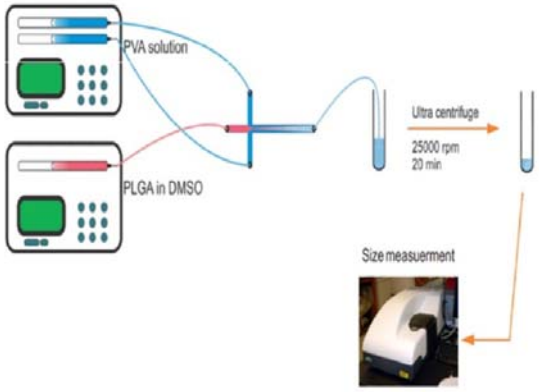

Fig. 1. The experimental setup. PVA aqueous solution is run in side inlets at different flow rates using two syringes on a syringe pump, and PLGA in DMSO is run in middle inlet using one syringe on a syringe pump. PVA, Polyvinyl alcohol; PLGA, polylactic-co-glycolic acid; DMSO, dimethyl sulfoxide.

Computational fluid dynamic simulation strategy

In this study, CFD modeling technique was used to investigate fluid flow behavior, mechanism and quality of mixing. For this purpose, three-dimensional geometries were created in Gambit pre-processing software, and the ANSYS FLUENT 15 software was used for simulating the microfluidic system. Computational domain consisted of two cylinders connected together. Different sizes and schemes of meshing were investigated to find suitable conditions, and regarding the time of calculation tetrahedral scheme was chosen as the most proper meshing method. The ratio of average PLGA concentration calculated by cells to the mean concentration of PLGA calculated manually at the outlet of microchannel was calculated to investigate the mesh study. There was no important change in the ratio of concentration for more than 780,000 tetrahedral meshes. Thus, this layout was applied to decline calculation time. Table 1 shows the mesh study for microchannel at flow rate ratios of PVA:PLGA solutions equal to 5 (R = 5).

In order to find the behavior of flow in microchannel, the Reynolds number (the ratio of inertial forces to viscous forces) was calculated by the following equation:

$$Re = \frac{\rho u d}{\mu} \quad (1)$$

where, ρ, u, d and μ are density (kg/m^3), velocity (m/s), diameter of microchannel (m) and viscosity (kg/m.s), respectively.

Re at different flow rates was calculated. Re numbers in all conditions were found lower than 2500; so, laminar flow was considered as the governing fluid flow regime. Also, PVA and PLGA concentrations in water and DMSO solvents were diluted, so fluids were considered Newtonian and velocity was supposed uniform at the inlet of channels. The gravity effect was ignored because the microfluidic channel was kept horizontally in all the experimental tests. The initial gauge pressure and temperature were adjusted 0 Pa and 300 K, respectively. The temperature difference was not significant, so the energy equation was not essential and assumed inactive in this simulation. Also, the properties of fluid including density, viscosity and diffusion coefficient were considered constant.

According to above assumptions, Navier-Stocks equations and Fick's second law for the performed system in this study can be written as follows:

$$\nabla . u = 0 \quad (2)$$

$$\rho u . \nabla u = -\nabla p + \mu . \nabla^2 u \quad (3)$$

$$\nabla(-D\nabla c) = -u . \nabla c \quad (4)$$

where, c is concentration (mol/L), μ is viscosity (kg/m.s), u is velocity (m/s), p is pressure (N/m^2), and D is the diffusion coefficient between two fluids (m^2/s).

Boundary conditions were set for inlets, outlet and walls as velocity inlet, pressure outlet and no slip wall, respectively. The standard SIMPLE algorithm was applied for coupling pressure and velocity and second order upwind scheme was adjusted for the mass transfer and momentum. The computation was continued until the solution converged to 10^{-6} for all variables.

Table 1. Calculation of ratio of PLGA concentration in the different number of meshes for the microchannel at R = 5.

Type of mesh	Number of meshes	Ratio of PLGA concentration
Hexahedral	3.1×10^4	0.741
Hexahedral	5.5×10^4	0.802
Tetrahedral	4.6×10^4	0.845
Hexahedral	7.2×10^4	0.884
Tetrahedral	5.9×10^4	0.957
Tetrahedral	7.8×10^4	0.995
Tetrahedral	8.9×10^4	0.998

PLGA, polylactic-co-glycolic acid.

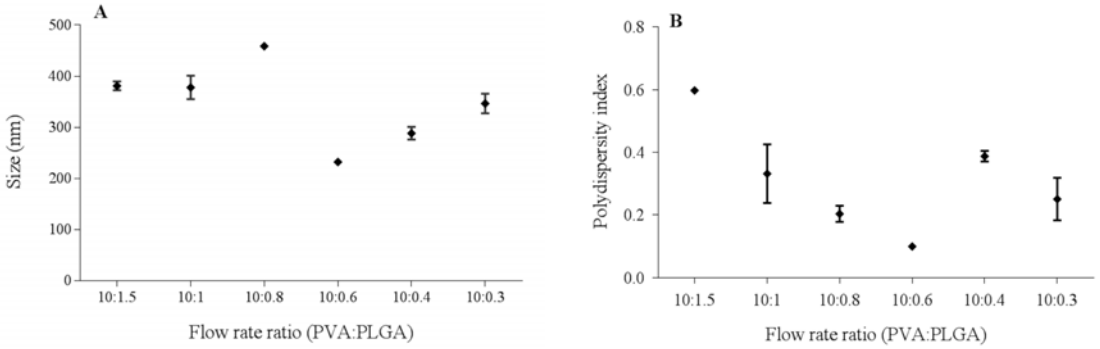

Fig. 2. Influence of flow rates ratio on the (A) size and (B) polydispersity index of nanoparticles.

RESULTS

Optimum ratio of flow rates

The particle size and dispersity in the microfluidic systems are tunable by adjusting the ratios of two solutions to find the optimum ratio. Nanoparticles were fabricated by injecting 2% PLGA and 1% PVA solutions to the chip. Different ratios were adjusted by changing the flow rate of PLGA solution and keeping the aqueous flow rate constant. The optimum ratio of injection obtained at flow rates of 10 and 0.6 mL/h for aqueous and organic solutions, respectively (ratio 10:0.6). According to Fig. 2, it is clear that at this ratio, particle size has the minimum value in comparison with other ratios. The optimum particle size and PDI of nanoparticles were found to be 238.1 ± 1.7 nm and 0.11 ± 0.015, respectively.

After achieving the best ratio of flow rates, all the remained experiments were accomplished using PLGA and PVA solutions at optimum flow rates (10 and 0.6 mL/h, respectively).

Our experimental result reveals that the size of nanoparticles obtained by the microfluidic method is tunable by changing the flow rates.

Computational fluid dynamic simulation and mixing quality results

As per the fact that micromixing of flows plays a vital role in mass transfer and preparation of nanoparticles in microfluidic system, CFD simulation was carried out for further understanding the flow behavior and mixing efficiency in the microfluidic system, and examining the effect of flow rates ratio on the mixing in channels. Three different flow rate ratios (1, 5, and 16.67) were chosen, and flow regime and mixing were observed at these ratios. The results corresponding to the contour of mass fraction of species together with velocity vectors of PVA and PLGA solution streams are illustrated in Fig. 3.

Mixing intensity at different flow rates ratios is compared in Fig. 4 with illustrating the velocity contour together with the velocity vectors at a surface located after confluence point. In this figure, all the three flow rate ratios have symmetrical vortices due to the presence of symmetry shape of microchannel. In R = 16.67, the vortices make more turbulence compared to other flow rate ratios and increase micromixing in the channel.

Beside the qualitative investigation of flow behavior in the microfluidic system, the micromixing was determined quantitatively by CFD for better understanding the mechanism of preparation within microfluidics. For this purpose, mixing quality was determined using following equation (21):

$$\alpha = 1 - \sqrt{\frac{\delta_M^2}{\delta_{max}^2}} \quad (5)$$

where, α is the mixing quality in the range of zero to one (zero indicates no mixing and one indicates perfect mixing), δ^2_{max} is the maximum variance of the concentration, and δ^2_M is calculated according to equation below:

$$\delta^2_M = \frac{1}{n}\sum(C_i - C_M)^2 \quad (6)$$

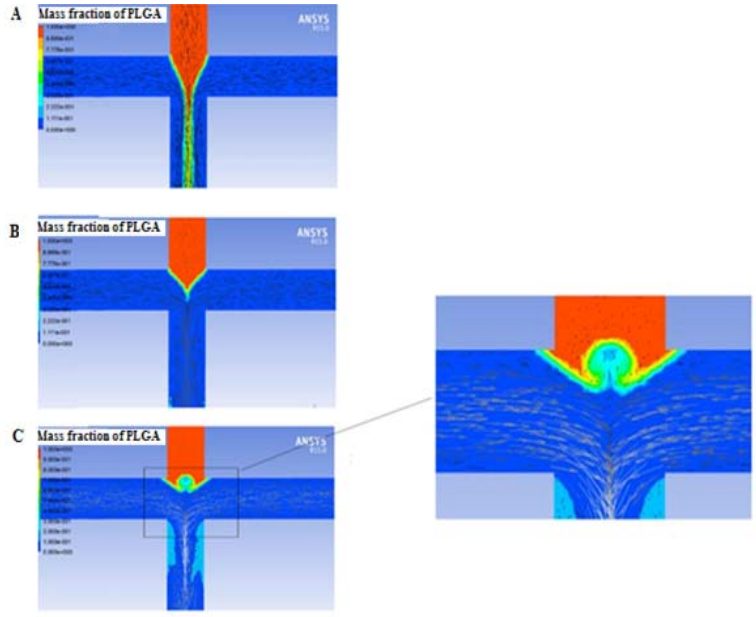

Fig. 3. The contour of mass fraction and velocity vectors of PVA and PLGA solution at different ratios (R) of PVA:PLGA solutions; (A) R = 1, (B) R = 5, and (C) R = 16.67. PVA, Polyvinyl alcohol; PLGA, polylactic-co-glycolic acid.

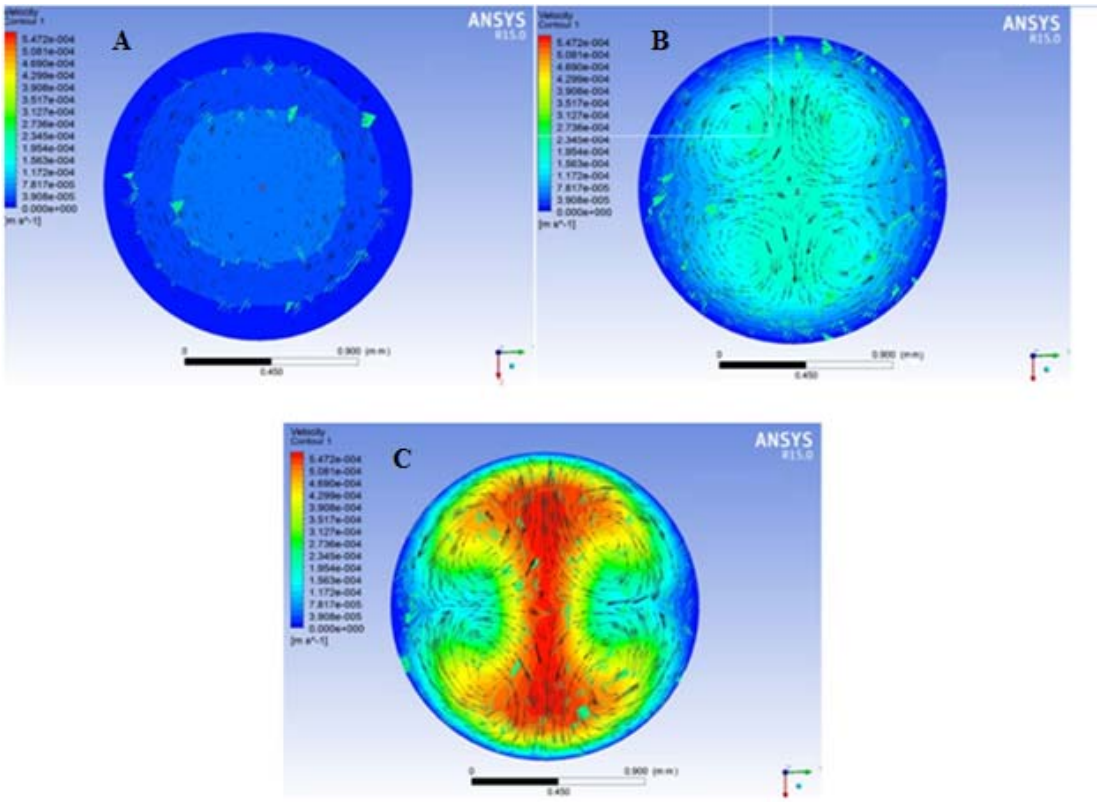

Fig. 4. Velocity contour and vectors at the surface close to the confluence point at different ratios (R) of PVA:PLGA solutions; (A) R = 1, (B) R = 5, and (C) R = 16.67

where, n is the number of nodes inside the cross section of the microchannel, C_M is the perfect mixing concentration, and C_i is the concentration value at node i in the cross section of mixing channel.

Mixing quality of the mixing channel at different flow rates ratio was calculated as a function of distance (x) from the beginning of mixing channel, and the results are plotted in Fig. 5. According to this Fig. 5, it can be seen that mixing quality increased during the length of channel because of micromixing. Two fluids entered the microchannel from inlets, joined together and mixing occurred in the mixing channel by small vortices depicted in Fig. 4.

The effect of polyvinyl alcohol concentration on the size and polydispersity index

The effect of surfactant concentration on the size and polydispersity of nanoparticles was studied using different PVA solutions (0, 0.0625, 0.125, 0.25, 0.5, 1, 1.5, and 2%), while the PLGA concentration was kept constant at 2%. The flow rates of PVA and PLGA solutions were 10 and 0.6 mL/h, respectively. The influence of PVA solution on the size and polydispersity index is depicted in Fig. 6. The results show that the optimum concentration is 0.0625% for PVA solution. As it is clear in the Fig. 6, ever increment in the PVA concentration causes increasing the mean diameter of nanoparticles.

Stability of nanoparticles during the time

In order to compare the results of the microfluidic method with the batch method, preparation of nanoparticles in the batch method at same operating conditions (2% PLGA and 0.0625% PVA) was conducted. The effects of time on the size and size distribution of nanoparticles, resulting from both methods, were studied. To evaluate the stability of nanoparticles, the particle size was measured after constant time intervals (1 h) and results are depicted in Fig. 7.

According to Fig. 7, it is clear that time has a negligible impact on the size and size distribution of microfluidic produced nanoparticles, but in the batch approach these changes over time are more than microfluidic, and the size of nanoparticles is affected by time much more than microfluidic system. Also, the standard deviation of diagrams in microfluidic is narrower than batch and microfluidic results are more repeatable than batch results.

Morphology of microfluidic and batch produced nanoparticles

The AFM images of obtained nanoparticles within microfluidic and batch methods at optimum conditions (2% PLGA and 0.0625% PVA) are shown in Fig. 8. It is obvious from the AFM image that nanoparticles obtained from microfluidic method are more uniform and monodisperse, and have completely spherical morphology, but nanoparticles acquired from batch method are polydisperse and not completely spherical.

DISCUSSION

This study for the first time attempts to provide a monodisperse, stable, and reproducible drug carrier as a candidate for delivery of different drugs in a controlled release manner, using microfluidic systems. Furthermore, CFD simulation was carried out in parallel to the experiments for better elucidating the flow behavior in such a miniaturized system. PLGA nanoparticles with proper size range and size distribution were produced successfully in the microfluidic chip. The precipitation was occurred rapidly due to the small volume of the microchip and efficient fast mixing, which causes generating homogeneous nanoparticles. Our experimental result revealed that the size of nanoparticles obtained by the microfluidic method is tunable by changing the flow rates.

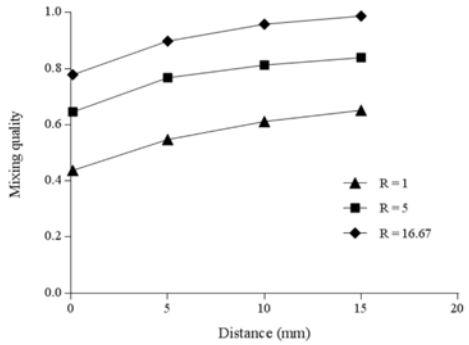

Fig. 5. The mixing quality of the mixing channel at different flow rates ratio (R = 1, 5, and 16.67).

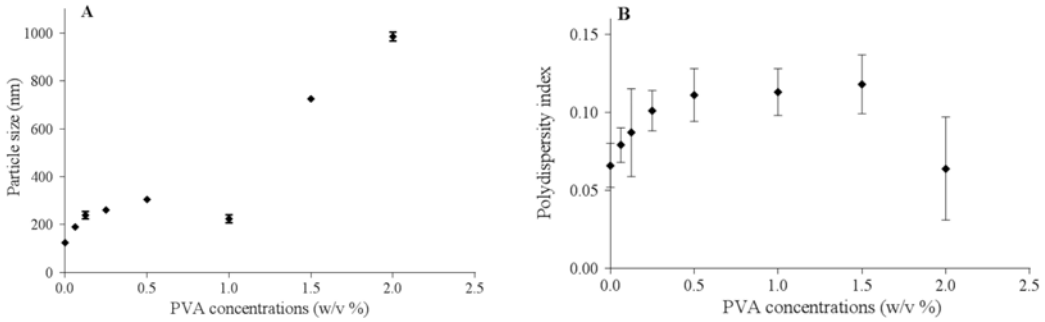

Fig. 6. The effect of PVA concentration on the (A) size and (B) polydispersity index of nanoparticles. PVA, polyvinyl alcohol.

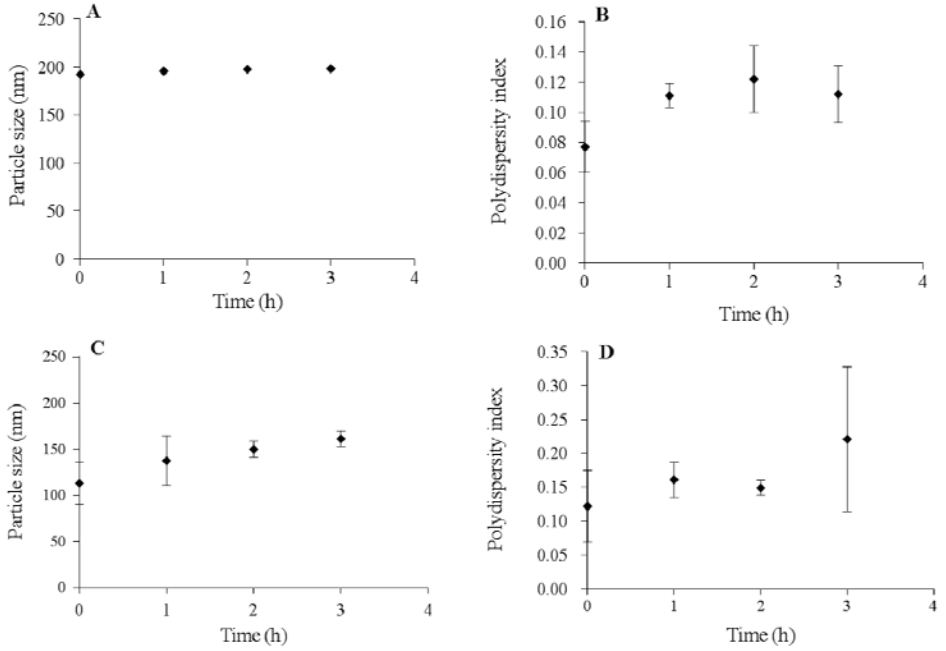

Fig. 7. The effect of time on the size and polydispersity index of nanoparticles in (A and B) microfluidic and (C and D) batch methods.

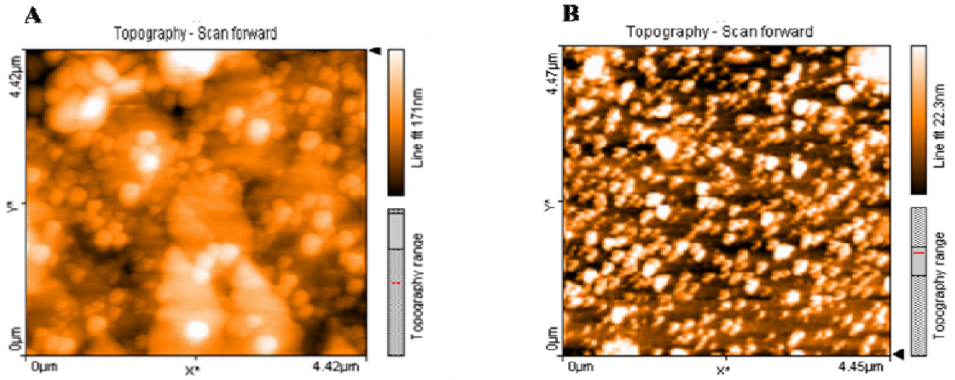

Fig. 8. The atomic force microscopy image of nanoparticles obtained by (A) microfluidic method and (B) batch method.

With increasing the flow rates ratio, both particle size and PDI, first decreased and then gradually increased. At constant flow rate of PVA (10 mL/h), decreasing the flow rate of PLGA solution from 1.5 to 0.6 mL/h caused a substantial reduction in the particle size and narrowing the size distribution, which is in good agreement with a former study, reported by Chang *et al.* (22). They reported a reduction in nanoparticles size with increasing flow rate ratio of external phase to internal phase due to decrease of the diffusion path for the existing particle. Increasing the flow rate ratio leads to narrowing confluent area (diffusion area) and reducing the diffusion path. Furthermore, the higher supersaturation can be achieved at higher flow rate ratio (10). The higher supersaturation results in formation of more nucleation sites and consequently fabrication of smaller particles and more uniform distribution of the particle size. Dirksen *et al*. proposed an equation to obtain crystal growth (23):

$$\frac{dl}{dt} = k_g(C_i - C^*)^b \quad (7)$$

where, dl/dt is the crystal growth rate, k_g is the crystal growth constant, C_i is the solute concentration on the crystal surface, C^* is the saturation concentration, and power b is a constant between 1 and 3.

It can be concluded that higher flow rate of aqueous solution, reduced the concentration of solute on the produced nanoparticle surface and resulted in fabrication of small nanoparticles due to a reduction in crystal growth rate. Another possible reason for the decreased particle size and PDI can be attributed to the micro-mixing intensity. It is arguable that mass transfer in microchannels is hugely affected by the intensity of mixing and turbulence. As the ratio of flow rates increases, the mixing intensity substantially increases due to strong impingement and robust turbulences and accordingly results in fabrication of small nanoparticles. The results disclosed the opposite trend by decreasing flow rate of PLGA solution from 0.6 to 0.3 mL/h. The reason could be imputed to the incomplete mixing of the solvent with the antisolvent (not enough residence time) in the mixing channel at higher flow rate ratios; so the solvent diffusion and transfer process could not be completed (24). The induced incomplete mixing at higher flow rate ratios may result in a non-uniform local supersaturation, thereby generating larger particles with wider size distribution. For further understanding the flow behavior at different flow rates, the mixing intensity at different flow rates ratios was compared using CFD simulation. There were significant differences in flow pattern at various flow rates ratios. At low flow rates ratio (flow rates ratio of 1:1) organic and aqueous phases flow in parallel layers with no significant mixing and visible significant interaction at the boundary. Increasing the flow rates ratio caused increasing the mixing intensity; thus, the layered streams were disturbed and mixing of two phases occurred immediately after confluence point (the center point of the channel). Furthermore, impingement velocity increases and vortices are formed, which cause efficient and dispersed fluid from the middle to the right and left sides of the mixing channel, decreasing the diffusion path, and consequently decreasing the size of nanoparticles. For the case with volume ratio of 10:0.6, robust vortices could be observed at confluence area. Although the robust mixing can be beneficial due to decreasing the size of nanoparticles, the robust impingement at contact point causes pushing a portion of organic phase flow back, and polymer is not distributed uniformly. This causes adverse effect on the preparation of nanoparticles and could cause fabrication of nanoparticles with different size in these areas. This effect is more significant for higher flow rates ratios (higher than 10:0.6), and it could be the chief reason for increasing PDI with increasing the ratios to higher than 10:0.6. Beside the qualitative investigation of flow behavior in the microfluidic system, the micromixing was determined quantitatively by CFD for better understanding the mechanism of preparation within microfluidics.

For all the cases, the mixing quality improves along the flow direction, and the highest mixing quality could be observed at the outlet of the channel. It is obvious

that flow rates ratio has a significant effect on mixing quality. At flow rates ratio of 10:0.6 the best mixing quality was achieved (about 0.99), which is in agreement with the visual results obtained by velocity contours.

The results show that the optimum concentration is 0.0625% for PVA solution. Although in absence of PVA the obtained nanoparticles had the best size and PDI, these nanoparticles were unstable, and particles could aggregate. The presence of PVA in solution caused more stable nanoparticles and prevented coalescing nanoparticles (25). So, minimizing the concentration of surfactant leads to achieving small particle size and narrow size distribution, and 0.0625% PVA solution was chosen as the best option.

Some inconsistent results for this effect have been reported in the literature. Zweers *et al*. (26) have found that at high PVA concentrations (5-10%) the size of PLGA nanoparticles increases, while Allemann *et al*. (27) reported that it decreases continuously. This contradiction can be attributed to the fact that at high PVA concentrations there are two competing effects. As the concentration of PVA increases, increasing the viscosity of solution leads to increasing the particles size, while increasing the interfacial stabilization results in a reduction of particle size. In this study, the high viscosity of aqueous phase had a dominant effect on the particle size. Due to aforementioned reason, in micro-scaled devices, the viscosity has a dominant effect on the flow characteristics and mass transfer within streams. It can be thought that increasing the viscosity of aqueous solution leads to hindering the diffusion between two solutions and thus results in non-uniform supersaturation and formation of larger particles.

The obtained particles were also examined in case of the stability and they were found to be sufficiently stable within the study period. However, the stability of the size of nanoparticles produced by batch method was less than that of nanoparticles produced by microfluidic system. Also, the results of microfluidic method are more reproducible than batch results. Reproducibility between batches was more difficult to achieve, which is generally in agreement with Khan *et al*. results (28). Considering the mean diameter of precipitated samples, the samples obtained in batch method were smaller, but the nanoparticles generated in microfluidic channel have lower polydispersity than those acquired by batch method. The high mixing intensity in microfluidic systems strongly affects the monodispersity of produced nanoparticles. Another reason indicating why particles produced by microfluidic chip are monodisperse is that in microchannels flow regime is laminar. Thus, system remains steady and flow characteristics remains constant. This can cause production of particles with similar shape and size. Also, this result indicates the greater stability of nanoparticles produced in the microfluidic method compared to the batch method.

It is obvious from the AFM image that nanoparticles obtained from microfluidic method are more uniform and monodisperse, and have completely spherical morphology, but nanoparticles acquired from batch method are polydisperse and not completely spherical. The AFM results were completely in agreement with Zetasizer results.

CONCLUSIONS

The current study provides an experimental study for determination of the particle size and size distribution of PLGA nanoparticles, obtained by the microfluidic-assisted nanoprecipitation method, and prepares a comprehensive study on the effect of process parameters on the size and size distribution of fabricated particles. It was concluded that increasing the ratio of flow rates of antisolvent to the polymer solution and performing antisolvent solution with low PVA concentration result in smaller nanoparticles. The best size and polydispersity index were obtained using 2% w/v PLGA, 0.0625% w/v PVA, and the PVA:PLGA flow rate ratio of 10:0.6. It was found that microfluidic devices can provide effective control of particle size and homogeneity. According to the CFD simulation results, the strong mixing intensity (the mixing intensity about 0.99 at optimum condition) in the microfluidic system can be

achieved, which is the possible reason for the advantageous performance of this system. Microfluidic method leads to the production of more homogenous, stable and tunable particles, thus the obtained particles can be taken into account as promising candidates for carrying different drugs in a slow and sustained release profile. Altogether, the results of the microfluidic method were found more reproducible and can be directly introduced from the lab to the industry without the need of scale-up.

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It must be mentioned Parisa Shokoohinia and Marziyeh Hajialyani contributed equally in this research.

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