The role of solvent in the formation of biodegradable polymer nanoparticles

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Abstract. Nanoparticles composed by the biodegradable polymers possess several advantages, i.e. improved stability of therapeutic agents against degradation, controlled drug release, and efficient targeting. The current investigation describes the detail of the formation and size control of biodegradable Polycaprolactone (PCL) nanoparticles obtained via nanoprecipitation process. A controlled millifluidic set-up is used to ensure the proper mixing of PCL molecules in the organic solvent to the aqueous medium (non-solvent). Small angle neutron scattering (SANS), dynamic light scattering (DLS) measurements are performed to extract the size distribution of PCL nanoparticles at different conditions. The analysis of DLS and SANS data reveal that droplets size is uniquely controlled by the solvent composition (acetone-water), which evolved during their gradual formation.

The use of biodegradable polymeric nanoparticles for controlled drug delivery has shown significant therapeutic potential. The biomedical application of polymeric nanoparticles strongly depends on the particle formation and their size [1]. Specifically, nanoparticles of 150-300 nm are mainly going to the liver and the spleen while particles of 30-150 nm can be found in the heart kidney and, stomach [2]. Therefore, the size and polydispersity of polymeric nanoparticles need to be controlled very precisely for clinical translation. Nanoparticles are prepared by using “nanoprecipitation” method, which is a simple, facile, mild and low energy input process to prepare the nanoparticles [3]. It consists of the addition of a hydrophobic compound dissolved in an organic solvent to form the diffusing phase. This phase is then added into an aqueous phase (non-solvent). Under these conditions, the precipitation of the polymer nanoparticles occurred spontaneously. This phase is the intermediate state of nanoparticles. The organic solvent is evaporated from the final solution in a second step and called the final state. In this study, we have investigated the drastic changes in the size of PCL nanoparticles along with changing the solute concentration and solvent to non-solvent ratio with the combination of DLS and SANS study. It is observed that with increasing the solute concentration, the nanoparticles size increases gradually. On the contrary, the evaporation of organic solvent reduces the size of the nanoparticles. The analysis of DLS and SANS results provides the fact that the changes in the PCL nanoparticles formation are associated with the solvent (acetone)-non-solvent (water) environment. The solvent composition controls the initial size of the nanoparticles and the growth as well [4,5]. These findings will certainly help to have the better control over the size and stability of polymer nanoparticles.

EXPERIMENTAL

Polycaprolactone (PCL) of $M_w = 14,000$ and acetone with 99.9% purity were purchased from Sigma-Aldrich. The polymer was received in the flakes form and used without any purification. D₂O (99.9 atom % D) was used to

ensure sufficient contrast for SANS experiments where H₂O was used for the DLS and Zeta Potential measurement. SANS experiments were carried out on the SANS-I facility at Swiss Spallation Neutron Source SINQ, Paul Scherrer Institute, Switzerland. All the measurements were performed at $\lambda = 8 \text{ \AA}$ with the wavelength resolution ($\Delta\lambda/\lambda$) about 10%. The raw data were radial averaged, corrected for electronic background and empty cell, and normalized by water scattering using the BerSANS software[6].

DATA ANALYSIS

DLS measurements were carried out using SZ-100 particle size analyzer (Horiba, Japan) at a wavelength of 532 nm. The diffusion coefficient (D_a) and polydispersity index (PI) are calculated using the cumulant analysis method. The effective hydrodynamic size (d_h) is calculated from D_a using Stoke-Einstein equation

$$d_h = \frac{k_B T}{3\pi\eta D_a} \quad (1)$$

where k_B is the Boltzmann's constant, η is the viscosity of the solvent and T is absolute temperature.

In SANS experiments, the scattering intensity in the absence of any interaction between the nanoparticles (valid approximation for dilute systems) for the particle of radius r and the polydispersity σ , with a log-normal distribution can be represented as

$$I(q) = N\Delta\rho^2 \int f(r) V^2(r) P(q, r) dr + B \quad (2)$$

where N is the number density of particles, $f(r)$ is the size distribution, and V is the particle volume. $\Delta\rho^2$ is the scattering length density difference of particle and solvent. $P(q)$ is the form factor and B is a constant term representing an incoherent background. $P(q)$ accounts for the scattering from a single particle and hence depends on shape and size of the particle. The interparticle structure factor is assumed to be one ($S(q) = 1$) for our system.

RESULTS AND DISCUSSION

PCL nanoparticles are prepared in a two steps method. In the first step, the polymer is dissolved in acetone and added to the non-solvent (water) by drop-by-drop using an infusion/withdraw syringe pump [5]. In the next step, the organic solvent is evaporated from the nanoparticles. In practice, 5.0 mg/mL PCL solution is prepared in acetone and added to water under stirring by a syringe pump. The speed of addition is kept fixed at 1.0 mL/min throughout the measurements. DLS results of a series of PCL nanoparticles intermediate states with an increasing number of added drops corresponding to increasing concentrations of polymer in the overall solution are presented in Figure 1a. The DLS results show differences in the decay of the intensity autocorrelation function. The decay of the intensity autocorrelation function of PCL nanoparticles at lower solute concentration is faster compared to PCL nanoparticles at higher solute concentration, which reveals the bigger nanoparticle formation with increasing the solute concentration in the nanoparticles. In the next step, a part of the polymer NPs is kept for overnight evaporation at the room temperature. DLS measurements are performed before and after the solvent evaporation. The intensity autocorrelation function obtained for PCL nanoparticles after organic solvent evaporation is presented in Figure 1b. It is assumed that no water is evaporated during the solvent removal in the room temperature. The solvent evaporation increases the solute concentration in the nanoparticles. Therefore, the swelling of nanoparticles can be expected as a result of growth after the organic solvent removal from nanoparticles. However, the results show the opposite trend. The intensity autocorrelation functions decay faster and the size of the nanoparticles decreases after the solvent evaporation. Another interesting result is observed that the intensity correlation functions are overlapped with each other except 2.42 mg/mL final concentration. The growth of nanoparticles by polymer addition cannot explain this phenomenon. Therefore, we have introduced the fraction of acetone as an independent parameter to control the size of the nanoparticles. In another study, it is observed for squalene nanomedicine that even after the organic solvent evaporation at low pressure and elevated temperature, a fraction of organic solvent can still present in the outer surface of the nanoparticles which decides the size and stability of the nanoassembly [5]. The size growth of polymer nanoparticles at different acetone fraction is presented in Figure 1c. The result clearly shows that with increasing the acetone fraction, the size of the PCL nanoparticles increased. On the other hand, the similar size obtained for the different final state suggests the presence of an equal fraction of acetone in the nanoparticles, which is verified by the weight measurement after the solvent evaporation.

FIGURE 1. DLS results of the (a) intermediate state of PCL nanoparticles obtained during the drop by drop addition of acetone solution (5.0 mg/mL) into H₂O. Inset shows the size variation of PCL nanoparticles with increasing the solute concentration. (b) final state of PCL nanoparticles at different concentrations after evaporation (c) size changes of the intermediate state of PCL nanoparticles in terms of the acetone fraction present in the solution.

In order to investigate the solvent-induced effect on the size of PCL nanoparticles in more details, we have performed a series of experiments where the aqueous environment (non-solvent) is tuned while keeping the initial and final concentration of the PCL fixed in the solution. For the DLS measurement, 0.065 mL PCL solution of concentration 1.01 mg/mL in acetone is added drop-by-drop to different mixtures of acetone and H₂O. The DLS results of PCL nanoparticles at different solvent compositions are presented in Figure 2a shows the differences in the decay of the intensity autocorrelation function. The decay of the intensity autocorrelation function of PCL nanoparticles without acetone in the non-solvent phase is faster compared to PCL nanoparticles dispersed in aqueous phase contains acetone. The decay becomes slower with increasing the acetone content in the aqueous medium for PCL nanoparticles reveals the bigger nanoparticles formation with increasing the acetone content.

FIGURE 2.(a) DLS and (b) SANS results of PCL nanoparticles at different acetone concentration in the aqueous phase.

To verify the trend observed by DLS measurements, SANS measurements are performed, where 0.1 mL PCL solution of concentration 5.0 mg/mL in acetone are added drop-by-drop to different mixtures of acetone and D₂O. The analysis of SANS spectra at large- q revealed that with increasing the acetone content the incoherent background is increased. At medium- q , the scattering intensity follows the q^{-4} scattering law (Porod law), which indicates the sharp interface of these objects [7]. The shifting of the Porod regime towards low- q with increasing acetone content is a clear signature for bigger nanoparticles formation. The Indirect Fourier Transformation (IFT) of SANS data confirms the spherical shape of PCL nanoparticles. The peak is shifted to higher- r with increasing the acetone content (Figure 3a) related to the bigger nanoparticles formation. The results obtained from DLS (hydrodynamic size) and SANS (particle size) data fitting are summarized in Figure 3b.

From the above study, the conclusion can be drawn that the solvent composition has an important effect on the size control of PCL nanoparticles. For nanoprecipitation processes, it is difficult to separate the effect of solute

concentration from the effect of solvent to non-solvent ratio, as both parameters are modified with the addition of solute drops (contains both the solute and solvent) to the non-solvent. Brick et al have followed a similar approach for the formation of organic cyanophenyl furanone colloidal dispersion by solvent shifting method and proposed the solvent/water counter diffusion, which is associated to a decrease in the solvent quality and leads to spinodal decomposition [8].

FIGURE 3.(a) Pair distance distribution functions $p(r)$ obtained from the fits of the experimental data. (b) Size analysis of PCL nanoparticles as a function of acetone volume fraction present in the solution.

The nanoparticle-nanoparticle interaction depends on the balance between the van der Waals attractive force and the repulsive electrostatic force due to the overlapping of electrostatic double layers. The negative zeta potential obtained for PCL nanoparticles reveals good stability of the nanoparticles. The electrostatic repulsion between surface charges of nanoparticles and $-\text{OH}$ ion dissociated from water prevent the aggregation arises from attractive van der Waals forces. With increasing the acetone content, the repulsive force decreases and the attractive van der Waal's force increases, which pushes the nanoparticles to coalesce and form bigger particles [9]. After a certain stage of coalescence, the global charge of nanoparticles is increased and the stability recovered.

CONCLUSION

PCL nanoparticles formation through the nanoprecipitation results from a dilution of the solute molecules in a non-solvent (water), causing the nucleation of very small aggregates of solute molecules, followed by the growth of these nuclei. The important message is that the solvent composition extensively regulates the size of these nanoparticles. Therefore, the control of the solvent composition has crucial importance for the size control and further biomedical translation of polymer nanoparticles. The idea remains to be consolidated by more extensive future studies with a series of biodegradable and conjugated polymer nanoparticles.

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