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Single-Step Microfluidic Synthesis of Various Nonspherical Polymer Nanoparticles via in Situ Assembling: Dominating Role of Polyelectrolytes Molecules

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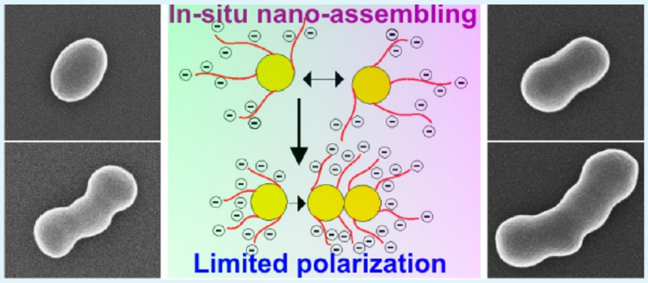
Supporting Information

ABSTRACT: In this paper, a microfluidic approach has been used for the synthesis of ellipsoidal, dumbbell, rodlike, and necklacelike polymer nanoparticles. High yields of special types of nonspherical nanoparticles have been achieved by the implementation of an emulsion polymerization into microfluidic arrangement with a micro hole-plate reactor for the formation of monomer droplets. Here, in particular, the formation of nonspherical polymer nanoparticles is dependent on the presence of polyelectrolyte surface active molecules such as poly(4-styrenesulfonic acid-*co*-maleic acid) sodium salt (PSS-*co*-PM), poly(sodium-*p*-styrenesulfonate) (PSSS), and polyanetholesulfonic acid sodium salt (PAES). The shapes and sizes of the interparticle nanoassemblies are precisely controlled by adjusting the concentration of polyelectrolytes in the aqueous phase, and by choosing suitable flow rate ratios (aqueous to monomer phase), respectively. The formation of polymer nanoparticles with different morphologies can be explained by a spontaneous in situ assembling under partial electrostatic repulsive control in the single step synthesis. The effect of particle charge and the competition between thermal motion of particles and electrostatic repulsion on the spontaneous assembling under the condition of a limited polarizability are discussed here as an important factor for the formation process of nonspherical polymer nanoparticles.



KEYWORDS: *polymer nanoparticles, nanoassemblies, ellipsoidal, dumbbell, microfluidic, limited polarizability*

1. INTRODUCTION

The deviation from a spherical shape means a symmetry breaking. In the case of nanoparticles, it is related to a considerable enhancement of surface energy because of higher surface to volume ratio of nanoparticles.[1] Therefore, it is of interest for obtaining new functional properties, special aggregation behavior or nano architectures, and realizing new materials. Nonspherical inorganic nanoparticles are formed, in particular, by forming spatial crystal shapes.[2] In contrast, polymer nanoparticles possess typically an amorphous character and forms as spheres. Spherical polymer nanoparticles can easily be formed by an emulsion polymerization.[3,4] The formation is controlled by a micelle mechanism if the concentration of surfactant is above critical micelle concentration (CMC), and when concentration of surfactant is below CMC, the mechanism is driven by homogeneous nucleation.[5] The particle size can be affected by surfactants concentrations and its types.[6] In general, a comparatively high homogeneity of particle size is achieved by this emulsification method. The emulsion polymerization is also well-suitable for the application of microreactors. Microreaction technology allows the realization of fast mixing of the reactants.[7] Moreover, they offer also best conditions for the efficient generation of emulsions from the mixtures of immiscible liquids.[3,8] Highly reproducible transport conditions[9] support a regular formation of nanoparticles of defined size. Microreaction technology was successfully applied, therefore, for the synthesis of inorganic dielectric nanoparticles,[10] semiconductor,[11] and metal nanoparticles[12,13] in micro continuous flow processes.

Here, the application of a microlithographically prepared micro hole-plate reactor for the generation of emulsion of the acrylate monomers in aqueous solution allowed a continuous-flow initiation of emulsion polymerization and resulted into high quality nanoparticles with better homogeneity. In this case, the synthesis of polymer nanoparticles succeeded without the application of a surfactant too, but at the end, particles are aggregated because of a lack of the inter-repulsive force. The particles diameter can be precisely tuned between about 30 and 600 nm by variation in concentrations of surfactant.[3,4] The surfactant has several functions during the polymerization process. At first, it is stabilizing the emulsion. Second, it can form micelles and stabilize the formation of polymer nano-

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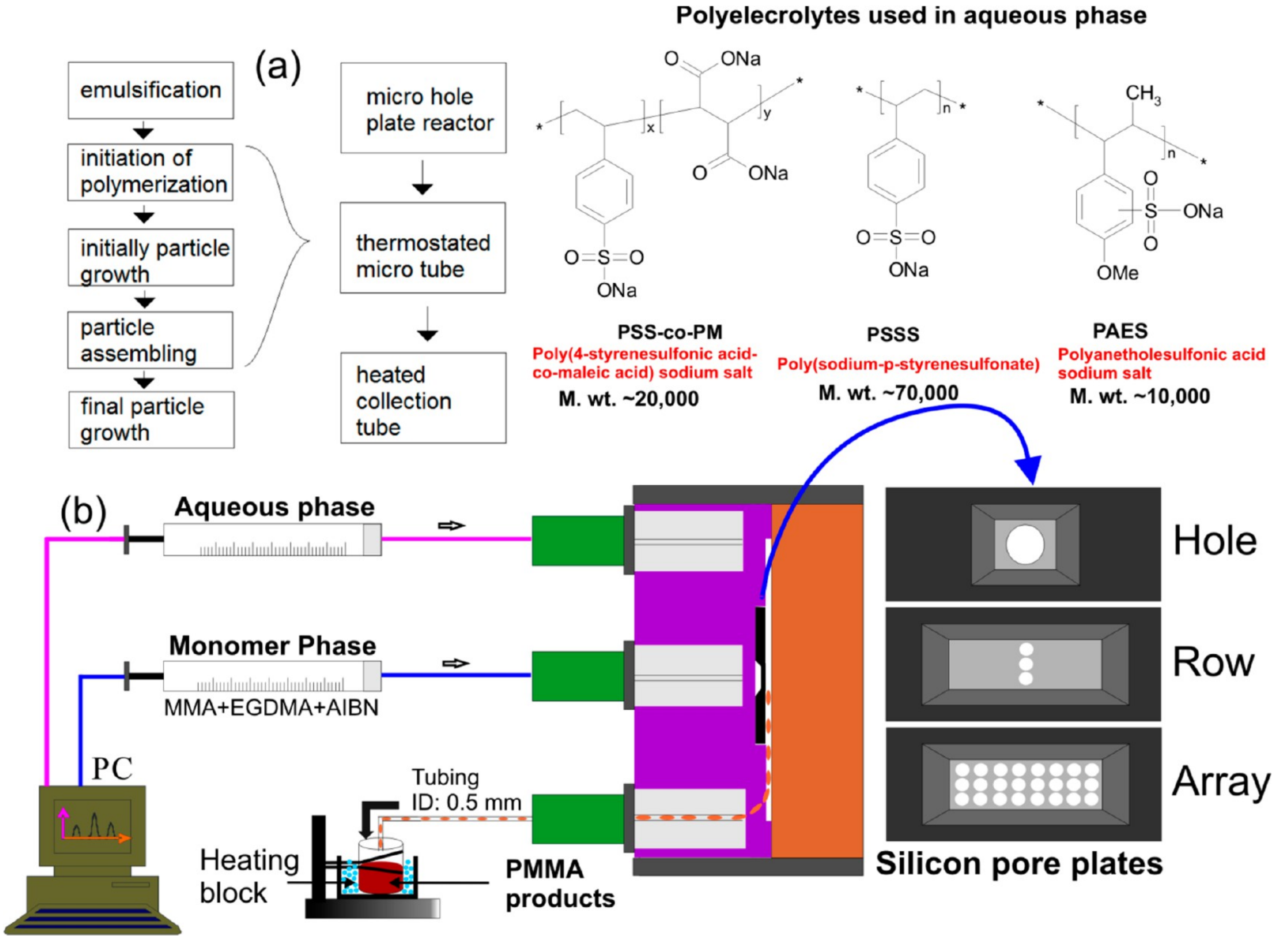

Figure 1. (a) Process concept of polymerization process and (b) a setup for the micro reactor arrangement.

particles. And third, it determines the interface properties of the forming polymer particles and, consequently, their interaction with solvent molecules, other solution components and with other particles. The last mentioned interaction is of particular interest because an irreversible interaction between nanospheres can be used for the generation of composed nonspherical nanoparticle types. The generation of differently shaped nanoparticles by particle/particle interactions is a general route for the synthesis of complex nanomaterials.[14,15] In the following, a new microfluidic method for the synthesis of different types of nonspherical polymer nanoparticles by an emulsion polymerization is presented and the formation of these particles is discussed by an electrically dominated mechanism for the control of in situ interaction of particles during their growth.

Polymer particles repeatedly have applications largely governed by their shape and morphologies.[16] In recent years, growing interests have been attracted for fabricating the colloidal polymer particles with anisotropic shape.[15,17,18] The defined shape is very important, which can be used as new carriers for drug delivery,[19] because the avoidance of uptake by immune cell is necessary to the survival of drug delivery carriers in the human body and is very sensitive to their shapes and morphologies rather than their sizes.[14,20] In photopolymerization, the arrangement and design of device or setup induces the required shape where anisotropic droplets are first generated and subsequently polymerized under the irradiation of UV light.[21,22] However, in such a type of arrangement, the generated droplets as well as particles are micrometer sized. The formation of nonspherical polymer nanoparticles at nanometer scale is very difficult via bottom-up synthetic approach. There are several reports where nonspherical shape have been attained via top-down or lithographic fabrication,[23] through solvent evaporation,[24] and through shape memory retention of block copolymer during the extrusion, embossing, or molding process.[25] However, in the above cases, the particles are obtained with inhomogeneous character. The syntheses of anisotropic polymer nanoparticles (consisting of an amorphous material) are very complex process, and such particles are hardly obtained by at least two or more steps (generation of seed particles in first step and subsequent growth in another one). Previously, it was claimed that the seed particles was a minimum requirement for the synthesis of dumbbell and other shaped polymer nanoparticles.[26,27] Interestingly, here we are presenting the formation of different types of homogeneous nonspherical polymer nanoparticles in a single-step micro continuous-flow process and explained them by an electrostatic control of in situ nanoassembling.

2. EXPERIMENTAL SECTION

2.1. Process Concept and Components of Reaction System.

One of the precious and general advantages of micro continuous-flow processes is the subdivision of a complex chemical process into separate steps, and the definition of optimal process conditions on the requirements of each individual step. In batch emulsion polymerization, all partial processes are performed simultaneously in one

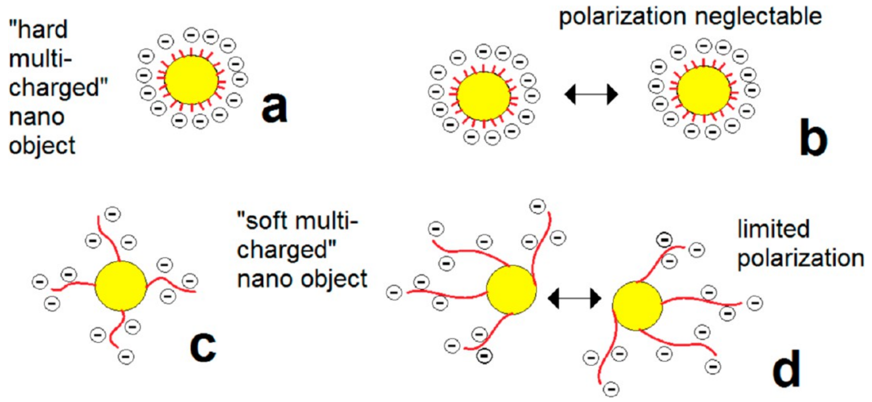

Figure 2. (a−d) Cartoons representing the scheme for the interaction of growing polymer nanoparticles and the role of adsorbed polyelectrolyte molecules.

reactor under the same conditions. In the micro continuous-flow process, the following individual steps are implemented in the different part of the micro reactor system (Figure 1a):

(a) Mixing of reactants at T-junction of microreactor.
(b) Generation of emulsion using a micro pore-plate reactor.
(c) Thermal initiation of polymerization by heat transfer in a flow-through heating block or heat exchanger.
(d) Completion of particle growth in a collector tube.

Syringe pumps (TSE systems, www.tse-systems.com) have been used for the fluid actuation. FEP (fluorinated ethylene propylene) tubes with an internal diameter (ID) of 0.5 mm were used for the fluid transport and for realizing the residence loop.

2.2. Micro Flow Emulsification and Polymerization Using a Micro Pore-Plate Reactor. The micro pore-plate reactor works by an injection of the monomer mixture through microlithographically prepared nozzles into the streaming aqueous solution. An aqueous phase is guided through a capillary slit in perpendicular direction to the monomer flow. Small droplets of the monomer liquid are breaks off by the strong shear forces in a cross-flow arrangement. The used pore-plate was prepared by a combination of time-controlled anisotropic crystallographic wet etching of silicon (100) for the main window and an ion beam etching of the micropores in a silicon membrane.[3] Pore plates with single pore (diameter 40 μm), with five pores (row arrangement, diameter 20 μm) and with 48 pores (12 × 4 array, diameter of pore is 5 μm) have been used in the synthesis experiments. The silicon chip with micropore was placed inside the microchannel assembly. A capillary slit with a width of about 0.2 mm was adjusted (in 2D manner) between the surface of the nozzle array and an opposite channel wall (Figure 1**b**). The Si membrane (1 × 2.6 mm^2) carrying the array was placed on a rectangular shaped chamber with sloped side walls inside the Si chip with an outer size of 1.6 × 3.3 mm^2 (picture shown in the Supporting Information, Figure S10). The shear forces have been varied by choosing the flow rate in the main channel (aqueous solution) between 260 and 2430 μL/min.

The formation of emulsion from two complementary phases started at the point of T-junction where monomer mixture meets a streaming aqueous phase of polyelectrolytes. Various concentrations of three different polyelectrolytes, poly(4-styrenesulfonic acid-*co*-maleic acid) sodium salt (PSS-*co*-PM), poly(sodium-p-styrenesulfonate) (PSSS), polyanetholesulfonic acid sodium salt (PAES) were used in continuous aqueous phase. One milliliter of monomer mixture contains 990 μL of methyl methacrylate (MMA) monomer, 10 μL of (1%) ethylene glycol dimethacrylate (EGDMA) cross-linker, and 3.5 mg of thermal radical forming initiator azobis(isobutyronitrile) (AIBN). Before the reaction mixture enters into a collection tube, the temperature of tube was set at 97 °C and therefore, the polymerization reaction started immediately after the reaction mixture came into contact with the heated tube. As a result, the polyelectrolytes stabilized size and shape controlled poly(methyl methacrylate) (PMMA) nanoparticles with various shapes such as elliptical, dumbbell, and necklace-type are obtained in dependence on the applied flow rate and polyelectrolytes concentration in aqueous phase. A similar reaction was performed in batch for confirming the role of microreactor for particles shapes. In batch experiment, no any homogeneous assemblies of linear shapes were obtained (without microreactor). Homogeneous elliptical and dumbbell shaped particles were obtained when PSS-*co*-PM polyelectrolyte was used in the aqueous phase. The concentrations of polyelectrolyte (PSS-*co*-PM) playing key role for in situ assembling of particles during growth, and therefore, at 0.09 mM PSS-*co*-PM (rep. unit conc.), the dumbbell-shaped particles with highest yield (about 90%) are obtained when flow rate ratio of 7.5 (300 μL/min/40 μL/min, aqueous/monomer) was applied. Similarly at 0.07 mM and at 0.05 mM PSS-*co*-PM concentrations (with same flow rate ratios), the dumbbell-shaped particles were obtained with about 70% yields. Moreover, polymer particles with ellipsoidal shape were obtained with about 80% yield at flow rate ratio of 70 (2220 μL/min/32 μL/min, aq./mon.) (conc. of PSS-*co*-PM was 0.09 mM).

2.3. Nanoparticle Characterization. *Scanning Electron Microscopy (SEM) Characterization.* All different kind of PMMA nanoparticles with different sizes and different shapes were characterized by SEM measurements. SEM images were obtained from Hitachi S-4800 and JEOL JSM-6380 instruments. For the preparation of samples for SEM investigation, the polymer nanoparticles were washed several times with deionized water by repeatedly centrifugation and redispersion process. One drop of particles suspension was deposited on silicon chip (5 × 5 mm) and dried at room temperature until evaporation of water naturally. To cover the surface of PMMA polymer particles, gold−palladium sputtering has been applied for 1 min, and then brought into the vacuum chamber of electron microscope.

Zeta Potential Measurements. The zeta potential of PMMA nanoparticles which have been prepared by using different concentration of different polyelectrolytes in aqueous phase were measured in Zetasizer nano zs (Malvern) instrument. All samples were prepared for zeta potential measurement in 1 mM NaCl (sodium chloride) solution. The particles were washed with deionized water six times to remove impurities prior to the measurements. Twenty-five microliters of particle solution was diluted in 1 mL of NaCl solution and filled in the transparent Zetasizer cell for obtaining the surface charge of the obtained particles.

3. RESULTS AND DISCUSSIONS

3.1. Concept of in Situ Nanoassembling by Potential Control in Emulsion Polymerization. Figure 1 show the process concept and microfluidic arrangement for the synthesis of nonspherical polymer nanoparticles. In general shape of the polymer particles obtained via emulsion polymerization is spherical, and a formation of this simple shape is caused by the minimization of interface energy. The interaction between the solvent (water) and the surfactants at an interface between the growing nanoparticle and the aqueous phase suppresses the direct contacts between the growing particles. In addition, an electrostatic repulsion between the particles leads to a further force, keeping the growing particles in distance from each other if ionic surfactants are used (Figure 2a). This electrical force is given by the type of used surfactant. The strength of electrical repulsion is determined by the density of surfactant molecules inside the liquid/liquid interface and by the number of charges per molecule. The electrical polarization of nanoparticles in case of an approaching other charged nanoparticle is low. It is derived by the field-induced deformation of particles or by pushing the charged surfactant molecules inside the liquid/liquid interface to opposite sides (Figure 2b). It is assumed that both effects are low in the case of surfactants with low molecular weight. Under these conditions, the polymer nanoparticles are "hard multi-charged nano-objects".

The situation concerning the electrical polarizability of growing nanoparticles is changed completely if surface-active substances with polyelectrolyte character are used. In this case, the mobile charged part of the molecules will be well-solvated by water and more or less stretched by the electrostatic repulsion between the single charged functional groups (Figure 2c). This results in a more or less "hedgehog" shape of the particles consisting of the polymer core and the radially stretched chains of surfactant macromolecules. If two of these particles are approaching, the charged chains of polyelectrolyte molecules will move to opposite directions in order to minimize the electrostatic repulsion (Figure 2d). This corresponds to a polarization of particles. The polyelectrolytes molecules behave as a polymeric soft micelles and assisting in the growth of the hard core polymer particles.[28] Nanoparticles with adsorbed poly ionic molecules behave as "soft multi-charged nano-objects". This polarization effects means also a reduction of repulsion in comparison with stiff objects with the same surface charge. This might cause a direct contact between two nanoparticles if their relative velocity overcomes the repulsive forces. Two cores can be united in case of an irreversible contact. This contact event can take place at different phases of nanoparticle growth during the emulsion polymerization. The shape of the formed particles will deviated to different degrees from a spherical one in dependence of the time of core assembling. Ellipsoidal particles will be formed in the case of earlier binding events (Figure 3). Dumbbell-like particle dimers are expected in the case of late binding events.

Ionic surfactants as well as adsorbed poly ionic macromolecules cause an electrical charge of the polymer nanoparticles. But, in contrast to metallic nanoparticles, the polymer particles connected with polyelectrolyte molecules can be polarized only by conformation changes of the adsorbed macromolecules, not by shifting electrical charges through the nanoparticle matrix (Figure 4a). This effect represents a "limited polarizability". The comparatively low molecular weights of the used polymers (between 10 and 70 kDa) correspond to chain lengths of the polyelectrolyte molecules between about 50 and 350 monomer units or sizes of the stretched molecules between about 30 and 200 nm. This is a little less or on the same order of magnitude as the diameter of the synthesized single spherical polymer nanoparticles or particle cores. The effect of limited polarizability determines the shape of assemblies in case of a second or further contact event between the growing particles, too. It reduces the probability of formation of compact assemblies because in this case the total of repulsion forces is particular high (Figure 4b).

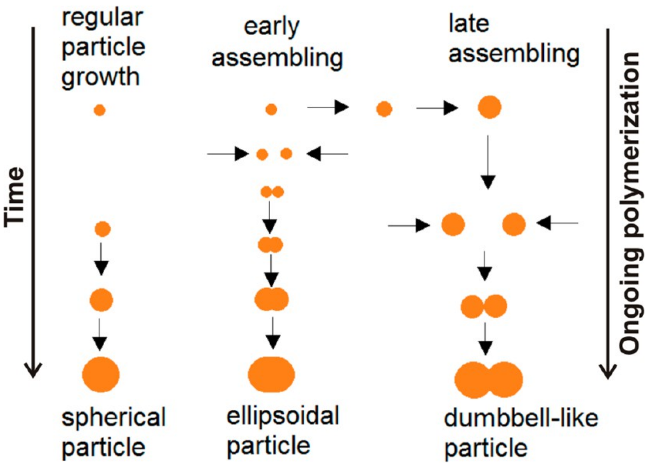

Figure 3. Principle of in situ dimerization and assembling of nanoparticles in different stages of their growth.

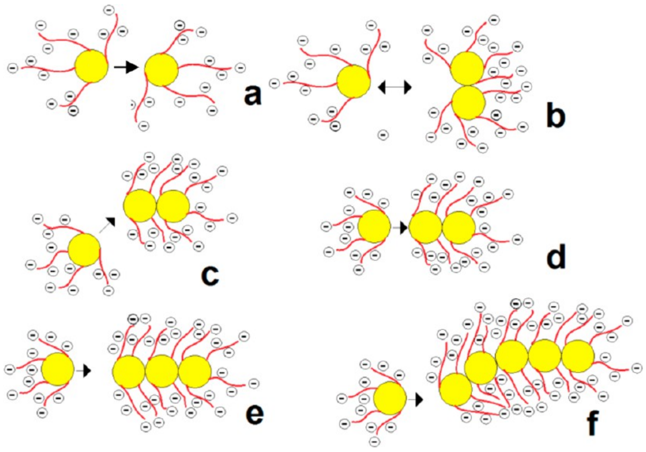

Figure 4. Assumed mechanism of formation of different types of nonspherical nanoparticles in case of in situ assembling of three or more polymer nanoparticles when lower aqueous to monomer flow rate ratio has been applied. (a−f) Assembling at different stages.

The total of repulsion forces reduced with increasing angle between the particle/particle axis (Figure 4c) and becomes minimal in the case of 180° arrangement (Figure 4d). As a result, the formation of assemblies with larger angles between the core particles or even completely stretched core arrangements has to be expected. These electrical effects influences further possible contact events and support a preferential formation of rodlike structures even in case of four or more nanoparticle cores (Figure 4e). The repulsion strength between the ends of such a chain of nanoparticle cores is lowering with increasing chain length. Therefore, the probability of angled arrangements increases with increasing length of the nanoparticle chain. Consequently, necklacelike nanoparticle assemblies (Figure 4f) should be formed.

3.2. Ellipsoid- and Dumbbell-Like Particles Obtained by Pairing of Growing Nanoparticles. At high flow rate ratios of aqueous solutions containing PSS-*co*-PM (0.09 mM) and the monomer liquid, the formation of ellipsoidal nanoparticles was observed, in general. It is assumed that these particles are formed by joining events between two spherical nanoparticles at an early phase of particle growth. The further growth of the particle dimers in ongoing polymerization leads to a compact but nonspherical final particle shape (Figure

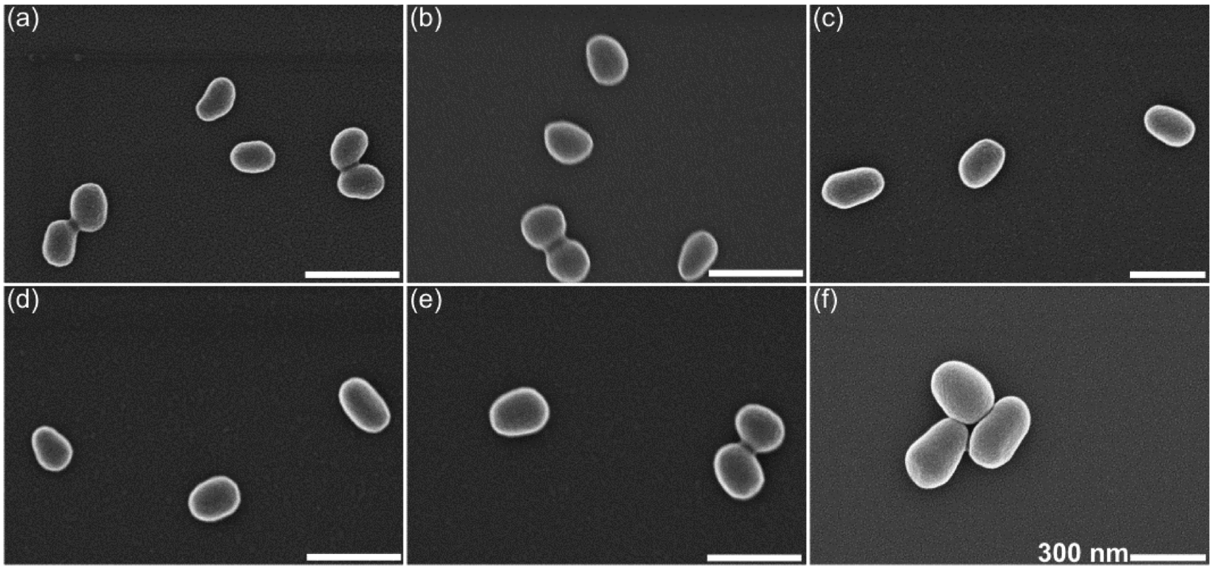

Figure 5. SEM images of the ellipsoidal polymer particles formed by early dimerization of spherical nanoparticles in the presence of PSS-*co*-PM. Effect of flow rate ratio and concentration of PSS-*co*-PM on the size of particles: 0.09 mM, (a) 3430/32 (aq/mon, μL/min), (b) 2230/32, and (c) 2030/32; 0.05 mM, (d) 2430/32, (e) 2230/32, and (f) 2030/32. Size becomes bigger when continuous aqueous phase flow rate is decreased.

5). The yield of these particles was comparatively high in all experiments. Only a few spherical particles have been observed beside the ellipsoidal particles. Obviously, the fast emulsification and the regular streaming conditions in the microfluidic arrangement support a fast initiation of polymerization as well as a synchronized growth of the particles. This results into homogeneous conditions for the formation of polymeric micelles and the development of a rather well synchronized growth of all nanoparticles. It is believed that the electrostatic conditions in all growing particles are comparable, which leads to similar conditions for electrostatic repulsion. This postulated homogeneity of conditions at the nanometer scale is probably the precondition for nearly exclusive formation of the ellipsoidal particles and the narrow distribution of particle shapes. The applied high flow rate ratios in the experimental series leading to the ellipsoidal particles that means the high density of polyelectrolyte molecules to nucleating nanoparticles. It is supposed that the strong Brownian motion of the small nanoparticles in the early stage of growth helps to overcome the repulsive forces and leads to the dimerization of small polymer cores. Furthermore, when a higher flow rate of polyelectrolyte solution in relation to the monomer flow rate is used, the density of macromolecules on the particle is enhanced, and therefore, the repulsion forces between particles are also enhanced. In general, nanoparticles require an input of energy or external forces to "direct" them into particular structure or assemblies.[29] Here, polyelectrolytes serve the controlled template for particle assembling which can be explained by the concept of limited polarizability. The high number of polyelectrolyte molecules at the liquid/liquid interface seems to prevent a further particle aggregation in the later phase of particle growth. The formation of the ellipsoidal particles is comparatively robust against deviations in the process conditions. The obtained aspect ratios shows only slight deviations if the flow rates and the surfactant concentration are changed moderately (Table 1). The higher number of polyelectrolyte charges causes a decrease in particles sizes. Thus, the formed particles are a smaller diameter of around 120 nm when 0.09 mM concentration of PSS-*co*-PM is used in the aqueous phase (flow rate is 3430 μL/min). With similar concentration of PSS-*co*-PM, the diameter of the particles slightly increased up to 130 nm when the flow rate of an aqueous phase is decreased down to 2000 μL/min at constant monomer flow rate. On other hand, the concentration of polyelectrolyte affects the particle size even at constant total flow rate. When three different concentrations of PSS-*co*-PM such as, 0.09, 0.07, and 0.05 mM were used, the diameter of the obtained particles are of 125, 130, and 150 nm at similar flow rate ratio, respectively. Both of these effects, flow rate ratio and concentration of polyelectrolytes, suggests an exclusive effect of polyelectrolyte charges to control the size of particles. The self-assembled aggregate of surfactants molecules in liquid medium is called micelles or vesicles. This micelles or vesicles forms naturally.[30] Interestingly, polymeric molecules also mimic the amphiphilicity and can self-assemble into polymer micelles or polymer vesicles.[31]

Table 1. Effect of Various Concentrations of Polyelectrolyte (PSS-*co*-PM, aq. phase) and Flow Rate Ratio of Both Liquids on PMMA Particle Size and Shapes

ellipsoidal shape regime			(aqueous phase)	dumbbell shape regime		
flow rate ratio (aq/mon, μL/min)	length (nm)	width (nm)	conc. of PSS-*co*-PM (rep. unit conc.)	flow rate ratio (aq/mon, μL/min)	length (nm)	width (nm)
3430/32	~150	~120	0.09 mM	800/40	~405	~210
2230/32	~160	~125	or	300/40	~450	~235
2030/32	~170	~130	(0.0030%)	260/40	~460	~240
2430/32	~155	~125	0.07 mM	360/40	~450	~240
2230/32	~180	~130	or	300/40	~470	~240
2030/32	~200	~140	(0.0023%)	260/40	~480	~250
2430/32	~170	~130	0.05 mM	360/40	~480	~240
2230/32	~200	~150	or	300/40	~500	~250
2030/32	~280	~200	(0.0017%)	260/40	~510	~260

The polymerization takes place at 97 °C. The polyelectrolyte molecule adsorbs and desorbs at the surface of growing particles consistently until the end of polymerization

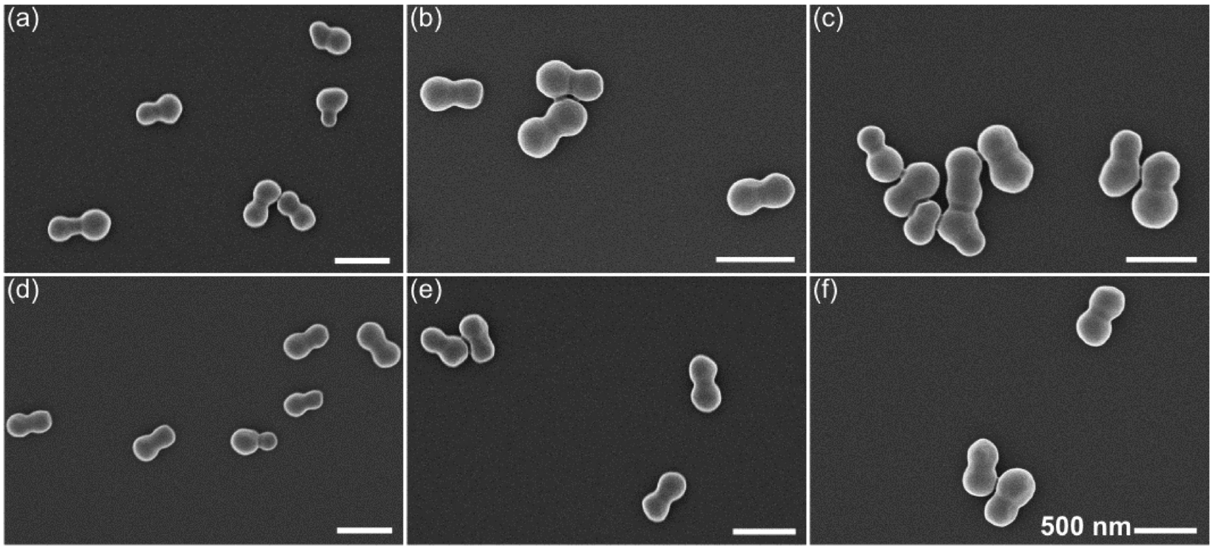

Figure 6. SEM images of dumbbell-like shaped particles formed by late pairing of growing PMMA nanoparticles in the present of PSS-*co*-PM. Effect of flow rate ratio and concentration of PSS-*co*-PM on the size of particles: 0.09 mM, (a) 800/40 (aq/mon, μL/min), (b) 300/40, and (c) 260/40; 0.07 mM, (d) 360/40, (e) 300/40, and (f) 260/40. Particle diameter increases with increasing continuous aqueous flow rate.

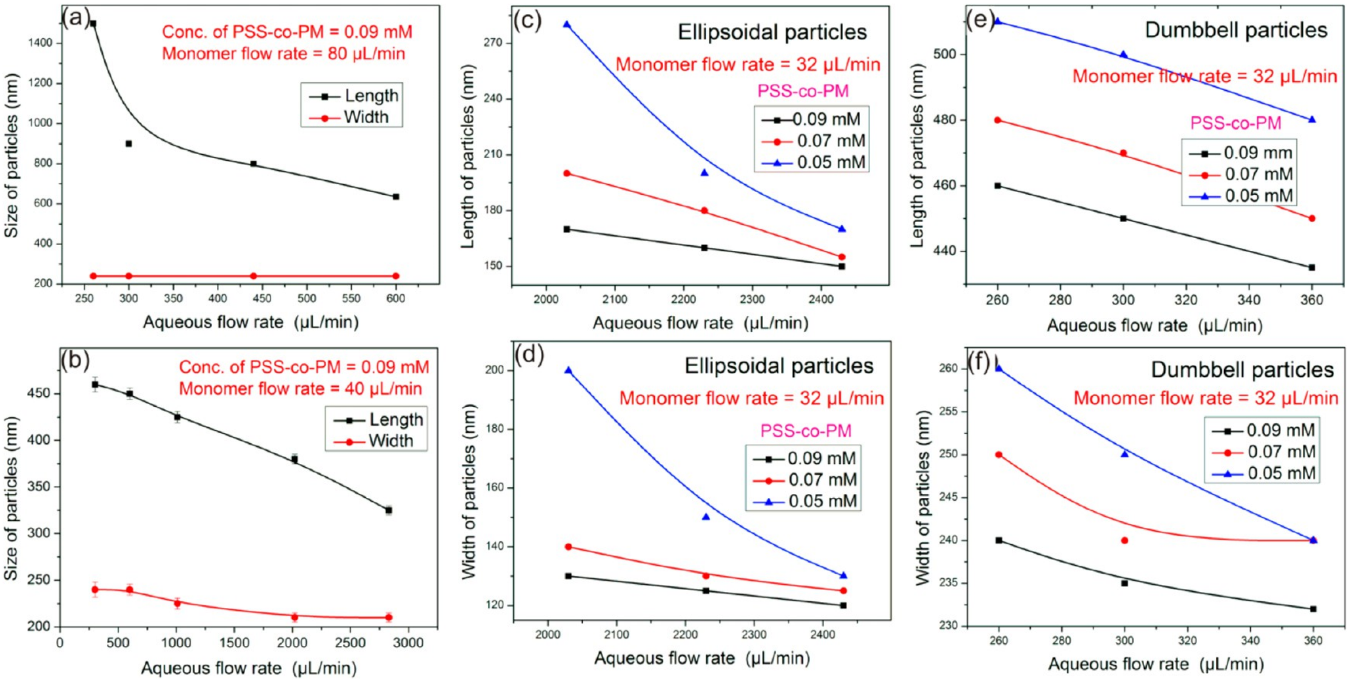

Figure 7. Graphical representation for the effect of different flow rates on the particles size: (a, b) change in particles size at different aq. flow rate when monomer flow 80 and 40 μL/min is applied, respectively; (c, d) change in length and width of ellipsoidal particles formed at very high aq. flow rate; (e, f) change in length and width of dumbbell shaped particles at lower aq. flow rate.

process.[5,32] During the dynamic process of adsorption and desorption of polyelectrolytes, methyl methacrylate (MMA) monomers enters into the polymeric micelle templates and, therefore, the growth of the particles obtained. As discussed above that ellipsoidal-shaped particles are obtained when higher numbers of polyelectrolyte charges are available on the surface (during higher concentration and higher flow rate of polyelectrolyte). In this case, there is no possibility for assembling third spherical particles to the ellipsoid, because in the limited polarization, all charges are gathered at both ends and, therefore, a high repulsion force is generated for the next adjacent particles in the solution. The results for the ellipsoidal particles are shown in Figure 5.

A significant other type of particle dimers (dumbbell) was formed if the flow rate ratio (aqueous/monomer) of both liquids was reduced drastically. The lowering of the flow rate of aqueous phase down to range between about 800 and 260 μL/min resulted into the formation of dumbbell-like particle dimers (Figure 6). It seems that a nonreversible joining of particles occur in the late phase of particle growth under these conditions. When a lower flow rate of aqueous phase (300 μL/min) has been applied, the polyelectrolyte charge density on the surface is comparatively low and, hence, comparatively lower suppression ability for the particle size. Therefore, at this stage, the final particle diameter is about 235 nm, almost double of the ellipsoidal particles. When the polyelectrolyte density on a surface is low, there is a competition at the early stage of polymerization between assembling of two cores and individual growth of a sphere. During the dynamic process of adsorption and desorption, the MMA monomer enters very quickly into the soft polyelectrolyte micelles template. This process realized by all individual growing spheres at same time into the ongoing polymerization reaction at 97 °C. Therefore, the growth of individual particles is preferred over the assembling event at very early stages. However, the assembly is obtained at the middle stage of growth as shown in Figure 3. Particularly at this stage, the charges are dense enough to repel the adjacent particles in the solution for further assembling event. Therefore, at the end controlled assemblies of dumbbell particles are obtained in high yield. Figure 7 represents the graphical results for the controlled sizes and shapes of ellipsoidal and dumbbell-shaped particles at different microfluidic reaction conditions.

An assumption from the effect of concentration ratio between the polyelectrolyte molecules and the growing nanoparticles on the electrical forces between the nanoparticles is well supported by measurements of the zeta-potential of obtained nanoparticles. In all cases a negative zeta-potential is observed which corresponds well with the negative charge of the polyelectrolyte components. This potential significantly increases in negative direction with increasing polyelectrolyte concentration (Figure 8). It increases slightly upward with decreasing flow rate ratio of aqueous and monomer phase. This finding indicates that not only absolute concentration of polyelectrolyte component is responsible for the decreasing zeta-potential, but also the number of polyelectrolyte molecules per nanoparticle.

3.3. Formation of Polymer Rods by Controlled Electrostatic Repulsion. Higher yields of aggregates of three or more polymer cores were obtained if the ratio of aqueous phase and monomer phase was lowered. Such larger assemblies have been formed typically at flow rates of the monomer phase between 80 and 100 μL/min and flow rates of the aqueous solution of polyelectrolytes (PSS-*co*-PM, 0.09 mM) between 300 and 600 μL/min. In all experiments, only a less number of compact aggregates were found. Obviously, in most cases angled assemblies (Figure 9a–c) or rodlike assemblies (Figure 9e–h) have been formed. This dominance of stretched over compact aggregates can be explained well by the assumption of electrostatic effects on the nanoparticle interaction. Compact particles of three or more cores can only be formed when electrostatic repulsion is much lower than the particle/particle affinity. In contrast, a significant repulsion and a polarization of one particle in case of the electrostatic field of an approaching second charged particle leads to the effect of "single-particle contact" as described above. The aggregation of three or more spherical particles was obtained when a comparatively high monomer flow rate has been applied. It means that the number of monomers to be entered into the polymeric micelle templates of polyelectrolyte is greater. The nucleation (initiation) of the particles immediately starts right after the emulsified droplet came into contact with heating atmosphere. A greater number of the nucleated individual particles are formed at the very early stage of polymerization, particularly in the case of higher monomer flow rate. Here, because of the lower density of electrical charges, monomer molecules quickly enter into the polyelectrolyte layer at the surface of particles. At the early stage in a dynamic polymerization process, small nucleated particles preferred to attach to larger, growing particles in the similar mechanism of the Ostwald ripening process.[33,34] The particles grow rapidly, and therefore the surface energy lowers. When there is a high density of polyelectrolyte available, the rate of monomer addition in the polymeric template is lower and in order to minimize the surface energy, smaller particles aggregate at an early stage of polymerization. However, the assembling is limited up to only two spherical particles, because then the polyelectrolyte chain gathers at both sides of the particles and prevents further assembling with additional spherical particles. Here, when higher monomer and lower polyelectrolyte are

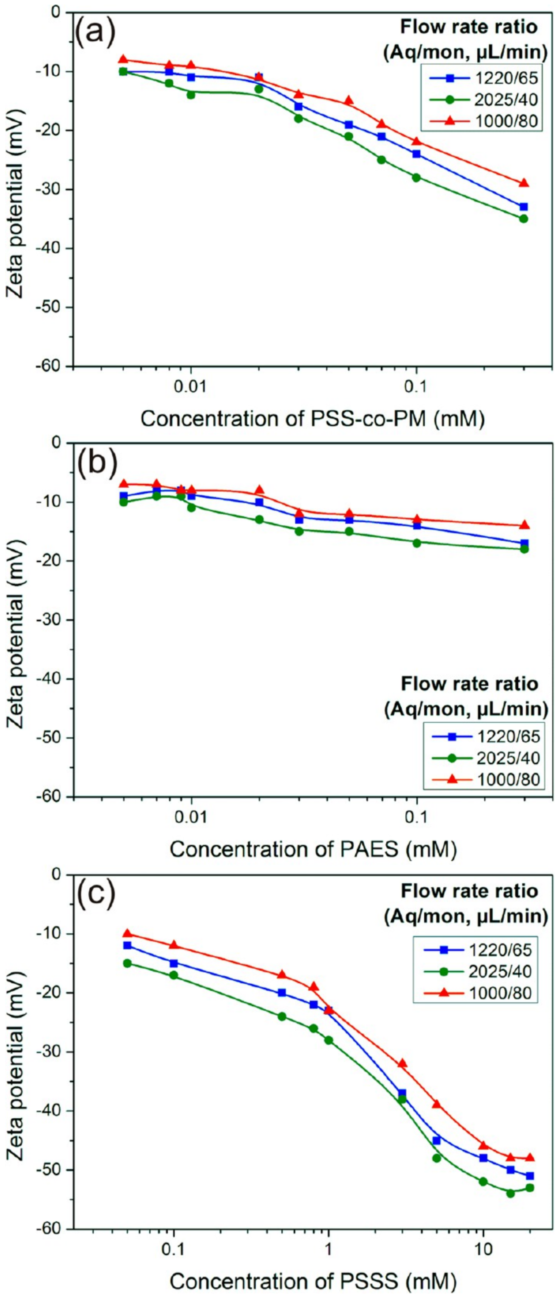

Figure 8. Graphical representation for dependence of the Zeta potential (final stage) of polymer nanoparticles suspensions on the concentration of (a) PSS-*co*-PM, (b) PAES and (c) PSSS at different flow rate ratio.

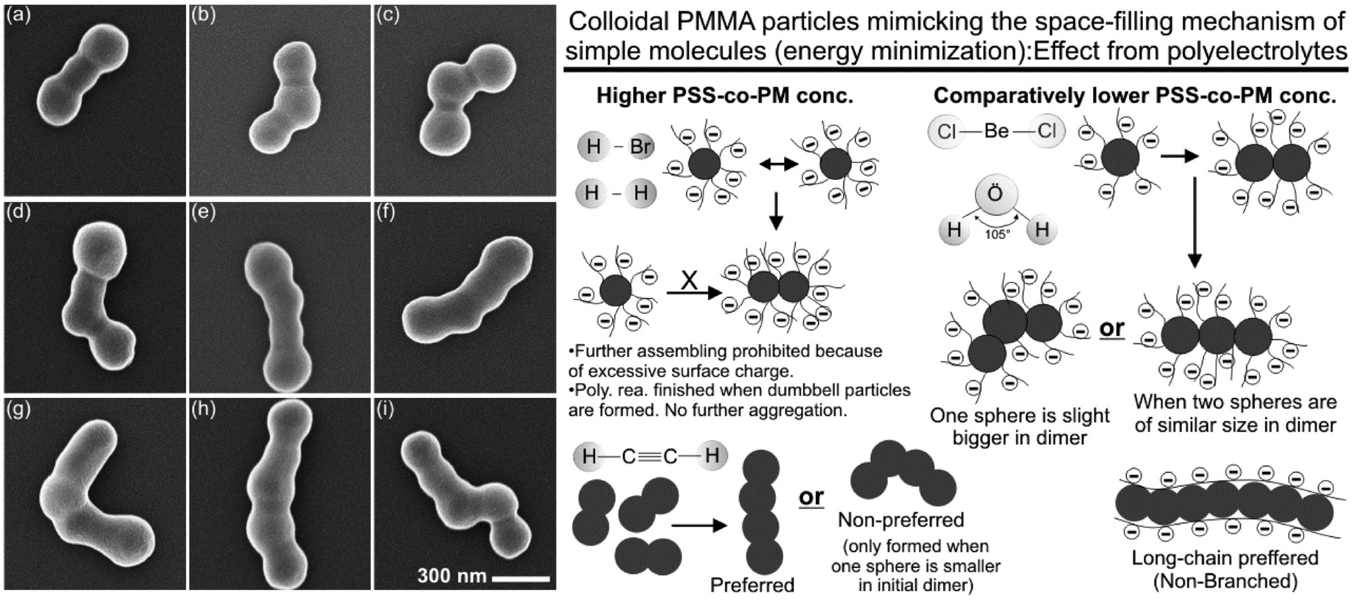

Figure 9. SEM images of the noncompact nanoparticle assemblies formed at lower flow rate ratios of surfactant-containing aqueous phase to monomer phase: (a−c) Nanoparticle assemblies formed by three core particles at 600/80 μL/min (aq/mon), (d−f) rodlike assemblies formed by four core particles at 440/80, (g−i) necklacelike particles formed by five core nanoparticles at 300/80. The concentration of PSS-*co*-PM in aq. phase is 0.09 mM. The scale bar is same for all images (300 nm). On right side, the proposed mechanism of particles assembly and final shapes that mimic the space-filling mechanism of colloidal molecules.

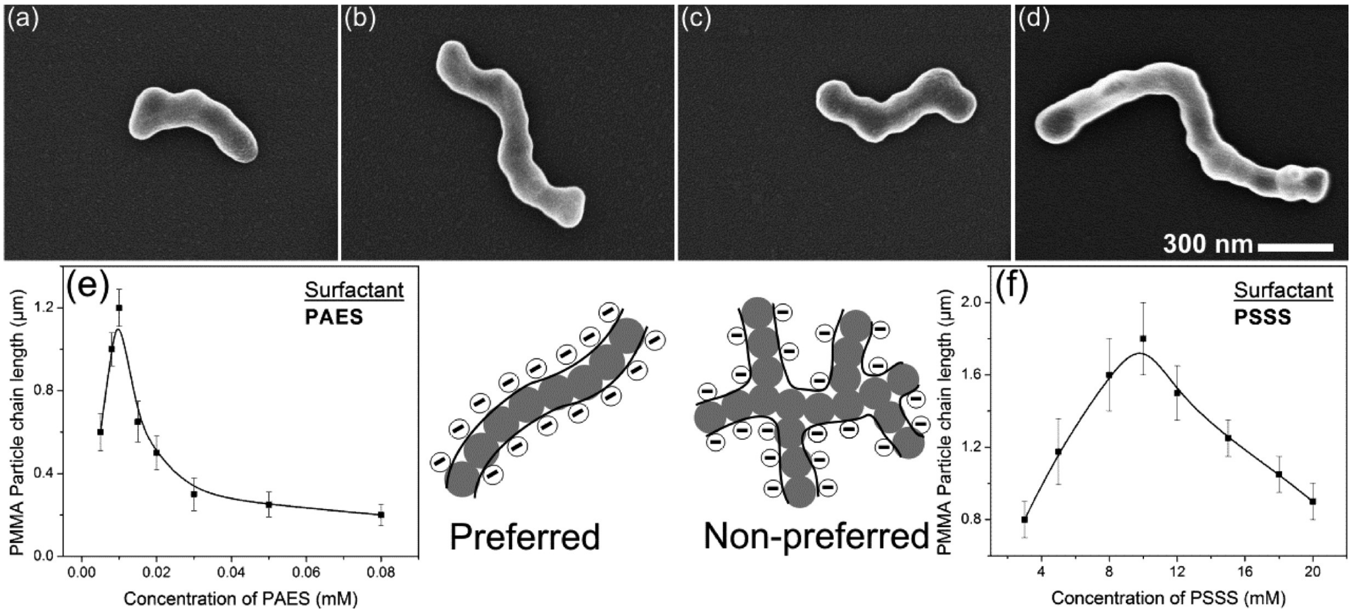

Figure 10. SEM images of necklacelike particles obtained when various concentration of PAES and PSSS polyelectrolytes were used in aqueous continuous phase: PAES, 2020/40 μL/min (aq/mon), (a) 0.015 and (b) 0.01 mM; PSSS, 1200/65 μL/min, (c) 20 and (d) 10 mM. The scale bar for all images is the same (300 nm). (e, f) Graphical representation of dependence of particle chain length on the concentration of PAES and PSSS.

present, two events happened. First, in the competition between individual spherical particle growth and assembling of two or more spheres at early or middle stage, the growth of the individual particles would be preferred. Second, spherical particles assembled at the late polymerization stage and three or more particles assembled to form the rod-shaped particles with high aspect ratio at different flow rate conditions, which is summarized in Table S1 (see the Supporting Information). The aggregation or precise clustering of the spherical particles in linear direction forms the particular shape of the final particles. The way of clustering, here, is dominated by energy minimization and, therefore the colloids adopts the shapes on the basis of mimicking space-filling mechanism of the simple chemical molecules, such as H_2O, CO_2, acetylene, etc. In other terms, the particles are so-called colloidal molecules.[35,36] Usually, the assemblies of the spherical particles are followed by the different routes of aggregation directed by physical or chemical route, self-assembly, coalescence, dominating forces, etc. The controlled clustering via depletion interaction is well-known,[29,37] but the phenomenon of the depletion is occurs whenever two different sized colloids or entities are presents in the liquids. In contrast to that phenomenon, here the

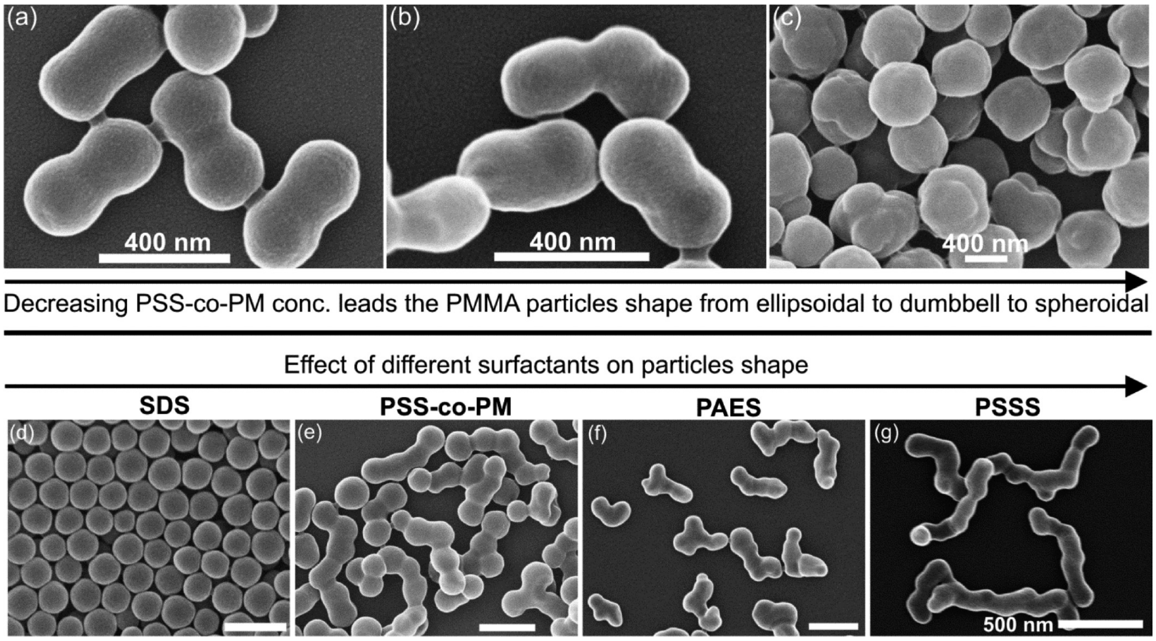

Figure 11. SEM images of the particles at different concentrations of PSS-*co*-PM (beyond the critical concentration where dumbbell shape formed): (a) 300/40 (aqueous/monomer), 0.05 mM, (b) 1220/65, 0.02 mM, and (c) 1220/65, 0.009 mM. (d−g) SEM images represent the different shapes of the particles obtained from the effect of different surfactant; SDS, PSS-*co*-PM, PAES, and PSSS, respectively.

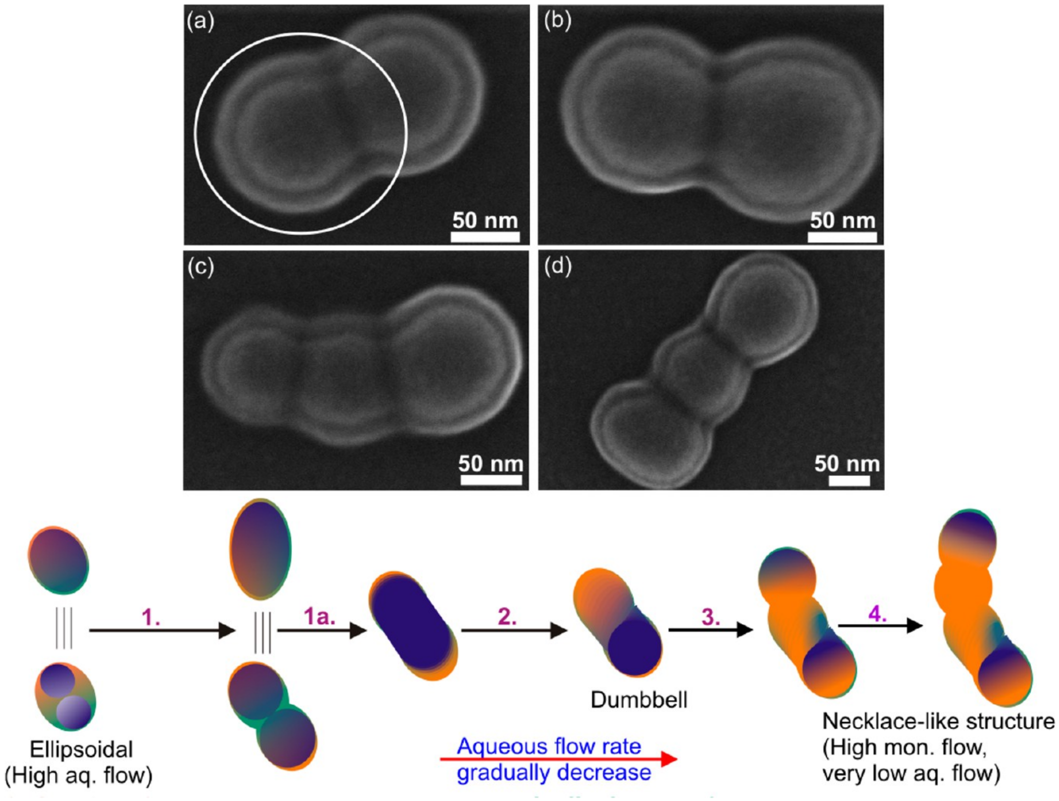

Figure 12. For confirming the assemblies of two or more spheres, SEM images of particles were taken after longer irradiation of electron beam inside the SEM vacuum chamber. By this, the top layer of polyelectrolytes melts and assembly of two and three spheres can easily be visualized: (a, b) two spherical particles are assembled and form dumbbell-shaped particles at meddle stage of polymerization reaction, (c, d) three spheres assembled and forms rod-shaped particles at the late binding event during ongoing polymerization reaction. Below cartoon shows the adoption of particles shape at different reaction conditions.

controlled assembling of homogeneous sized particles obtained during the ongoing polymerization reaction. The adoption of the final particles shape is governed by the dominance of energy minimization, and the entire assembling process in a precise manner is depends on the polyelectrolyte templates (with different chain-lengths and different concentrations).

3.4. Nanoparticle Chains Obtained by Late Aggregation. At lower flow rate ratios, and in particular if PAES and PSSS were used, a higher tendency to assemble more than three particles was observed. Sometimes, about a dozen particle cores are connected (Figure 10). The necklacelike shapes indicates their formation in a late phase of the polymerization process. It seems that the attachment of a new polymer core on a pre-existing chain is always preferred on the polar positions or at the ends of the particle chain. The preferential binding of new nanospheres at the ends of a chain can be explained by the model of limited polarizability as discussed in above section. The characteristic length scale of polarization is on the order of magnitude of one nanoparticle sphere or a little below it. Therefore, an electrostatic effect of a spherical particle approaching a pre-existing rodlike or necklacelike assembly acts only locally. The response in the conformational motion of the adsorbed polyelectrolyte molecules guides the approaching nanosphere to the ends of the chain. There are best boundary conditions for pushing away a part of the negative charges and, therefore, the lowest electrostatic threshold for an attachment of the spherical nanoparticle available on the pre-existing chain. The long necklacelike assembly is formed, particularly when linear polyelectrolytes such as PAES and PSSS are used. Particularly, controlled ellipsoidal and dumbbell-like particles are formed during the utility of copolymeric polyelectrolyte, PSS-*co*-PM.

In all particle shapes explained above where the role of polyelectrolytes was proved dominating, the further impact of their nature and their concentration can create even a broad spectrum of the size and shape library. The critical concentration of PSS-*co*-PM for achieving homogeneous dumbbell-shaped PMMA particles is 0.09 mM. Beyond the higher and lower limit of this critical concentration, there is a change in particles shape. Therefore, when PSS-*co*-PM concentration in the aqueous continuous phase decreases step by step, the final shape of particles is transformed from dumbbell to deformed dumbbell to aggregated spheroidal-like. Spherical shape is the usual form in emulsion polymerization. Therefore, at 0.009 mM PSS-*co*-PM concentration, the shape is not assisted by the template of polyelectrolytes. This lower concentration (0.009 mM) is not sufficient to form the polymeric micelle, and therefore inhomogeneous spherical shaped particles obtained (in general shape with lowest surface energy) (Figure 11). Short-chained ionic surfactant forms the micelle structure and led the spheroidal shape of particles. PSSS, PAES and PSSS impose the special characteristic for shape evolution in controlled manner (Figure 11d–g). At the end, to confirm the particle assembly inside the polyelectrolyte templates, the dumbbell- and rod-like-shaped polymer particles reamin under the influence of electron beam in SEM vacuum chamber for a longer time (Figure 12).

Despite possessing an amorphous nature, here, the morphology of the polymer particles can easily be controlled in a reproducible manner with the help of microreaction technology. Utilization of the different concentration of individual and mixed fluorescent dyes during the single-step synthesis makes the particles fluorescently active. These fluorescent nonspherical particles tune the florescent properties, and can find their application in the microtoxicological screening experiments on living organisms. The test experimental results of fluorescence measurements of particles are shown in Figure S12 (see the Supporting Information). Compared to spherical particles of same size, the surface to volume ratio of the obtained particles are very high, and therefore, these linear particles can give an intense effect for microtoxicological screening of living organism. Moreover, the obtained polymer particles can find their application for preparation of anisotropic polymer–metal nanocomposites. The model experimental result is shown in Figure S13 (see the Supporting Information). A previous study revealed that the avoidance of uptake by immune cells is necessary to the survival of drug delivery carriers in the human body and is very sensitive to their shapes and morphologies rather than their sizes.[20] Furthermore, the shapes of the obtained polymer particles are ellipsoidal and linear rodlike, and therefore, they mimic the structure of the *Escherichia coli* (polymer rod) and *Staphylococcus aureus* (polymer ellipsoid) during an in situ segmented flow characterization in miniaturized bioscreening for the enhancement of fluorescent and optical properties after the assembling with functional plasmonic and dielectric materials.

4. CONCLUSIONS

The investigations show that the microfluidic concept of emulsification of monomer liquid by micro hole-plate reactor can be advantageously used for the synthesis of nonspherical polymer nanoparticles. It was possible to achieve a shape spectrum from ellipsoidal and dumbbell-shaped nanoparticles to rod- and necklacelike nanoassemblies. Here, different shapes of the polymer nanoparticles have been well-addressed by the variation in types and concentrations of the polyelectrolyte molecules and by varying the flow rates of the aqueous and monomer phases. In contrast to other assembling processes of nanoparticles, the probability of formation of compact assemblies was low, whereas linear assemblies dominated in the case of aggregation of more than two spherical nanoparticles. The formation of the different obtained shapes can be explained by electrostatic repulsion effects and a "limited polarizability" of nanoparticles caused by conformational changes of the adsorbed polyelectrolyte molecules. The interpretation of the effect of polyelectrolyte molecules on the nanoparticle shape is well-supported by the zeta potential measurements and SEM images.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

SEM images of extra results of nanoassembling, tabular result of particles aspect ratio at different reaction condition, and effect of different surfactants on particle morphology. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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