SUPPLEMENTARY INFORMATION

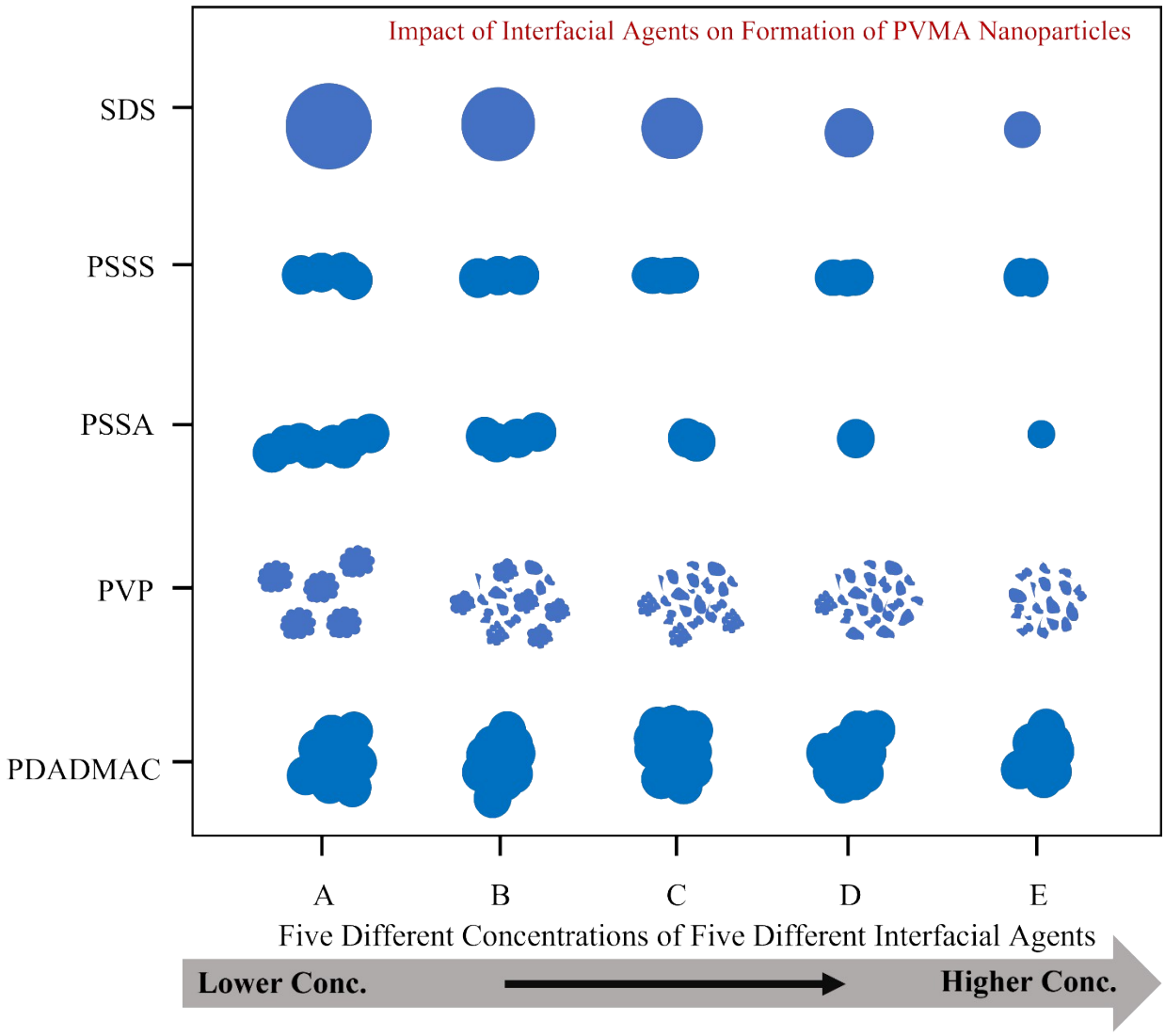
Microfluidic-Supported Synthesis of Anisotropic Polyvinyl Methacrylate Nanoparticles via Interfacial Agents.

Nikunjkumar R. Visaveliya[a], Seda Kelestemur[a,b], Firdaus Khatoon[a], Jin Xu[a], Kelvin Leo[a], Lauren St. Peter[a], Christopher Chan[a], Tatiana Mikhailova[a], Visar Bexheti[a], Ashni Kapadia[a], Piyali Maity[a], William P. Carbery[a], Kara Ng[a,c], and Dorthe M. Eisele[a,c]*

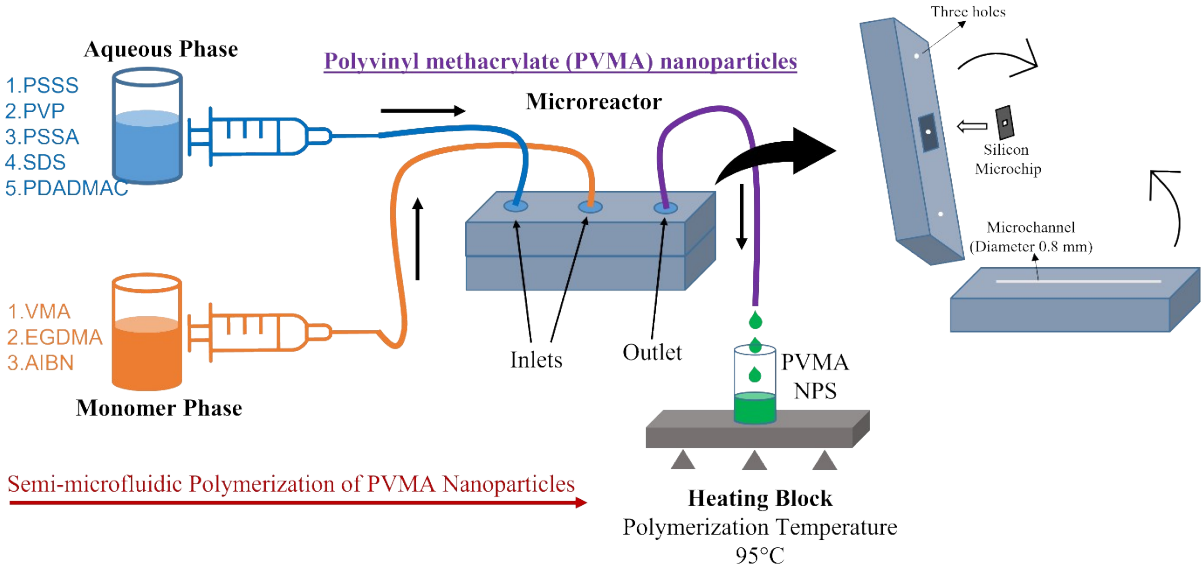
[a]Department of Chemistry and Biochemistry, The City College of New York at The City University of New York, USA; [b]Biotechnology Department, Institute of Health Sciences, University of Health Sciences, Istanbul, Turkey; [c]Ph.D. Program in Chemistry, Graduate Center of The City University of New York, USA a. Department of Chemistry, The City College of New York at The City University of New York, USA

*Corresponding author: eisele@eiselegroup.com

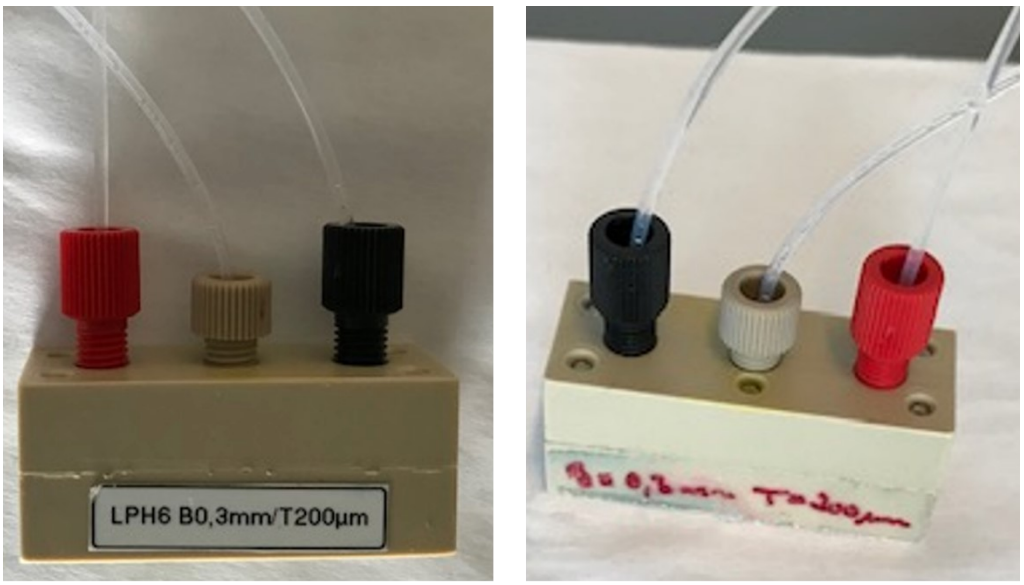
Table of Contents



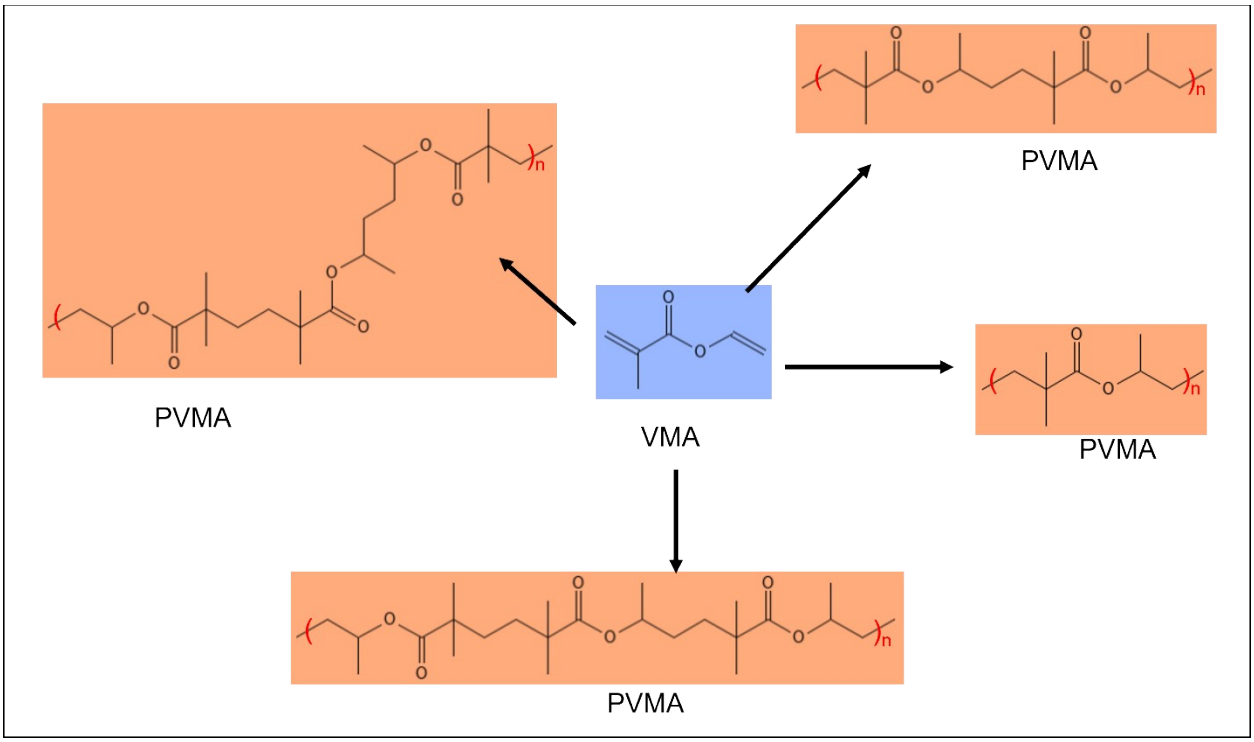
Overview of the shapes of PVMA nanoparticles during the application of various types and concentrations of five interfacial agents.



A general arrangement of the semi-microfluidic setup for the polymerization to form PVMa colloidal particles. The schematic details about the microfluidic reactor are also given (right part).



A photograph of the microreactor used during our experiments.

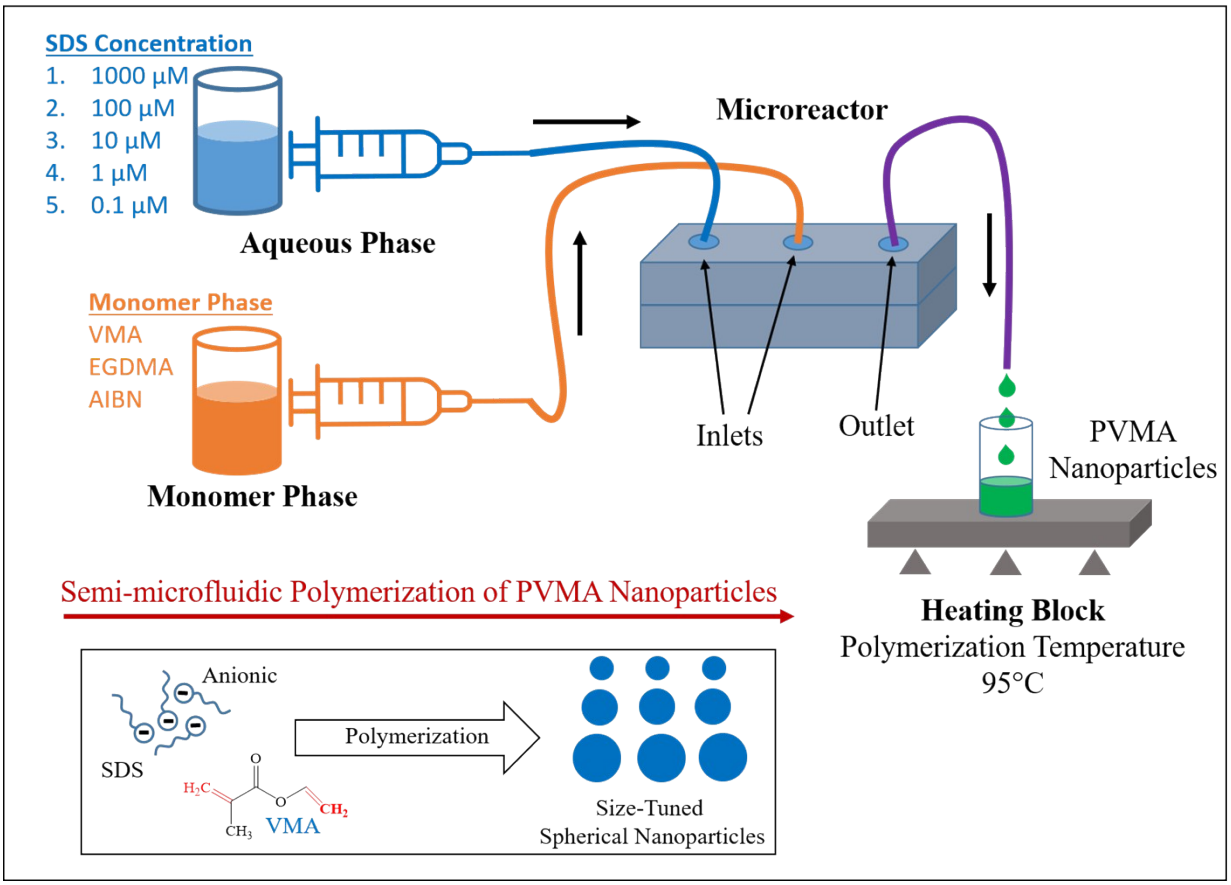


A general possibilities of the polymeric structures of PVMA after polymerization of VMA monomers.

1. PVMA Nanoparticles: *Impact of SDS*

The size of the spherical PVMA nanoparticles (obtained during the application of SDS surfactant in the aqueous phase during polymerization) has been measured via SEM characterization. The manual measurement of the size overview of the spherical nanoparticles was obtained during the SEM measurement. 40 spherical nanoparticles were measured from four different SEM images of the same sample (10 nanoparticles from each image) and an average size with its standard deviation has been obtained.

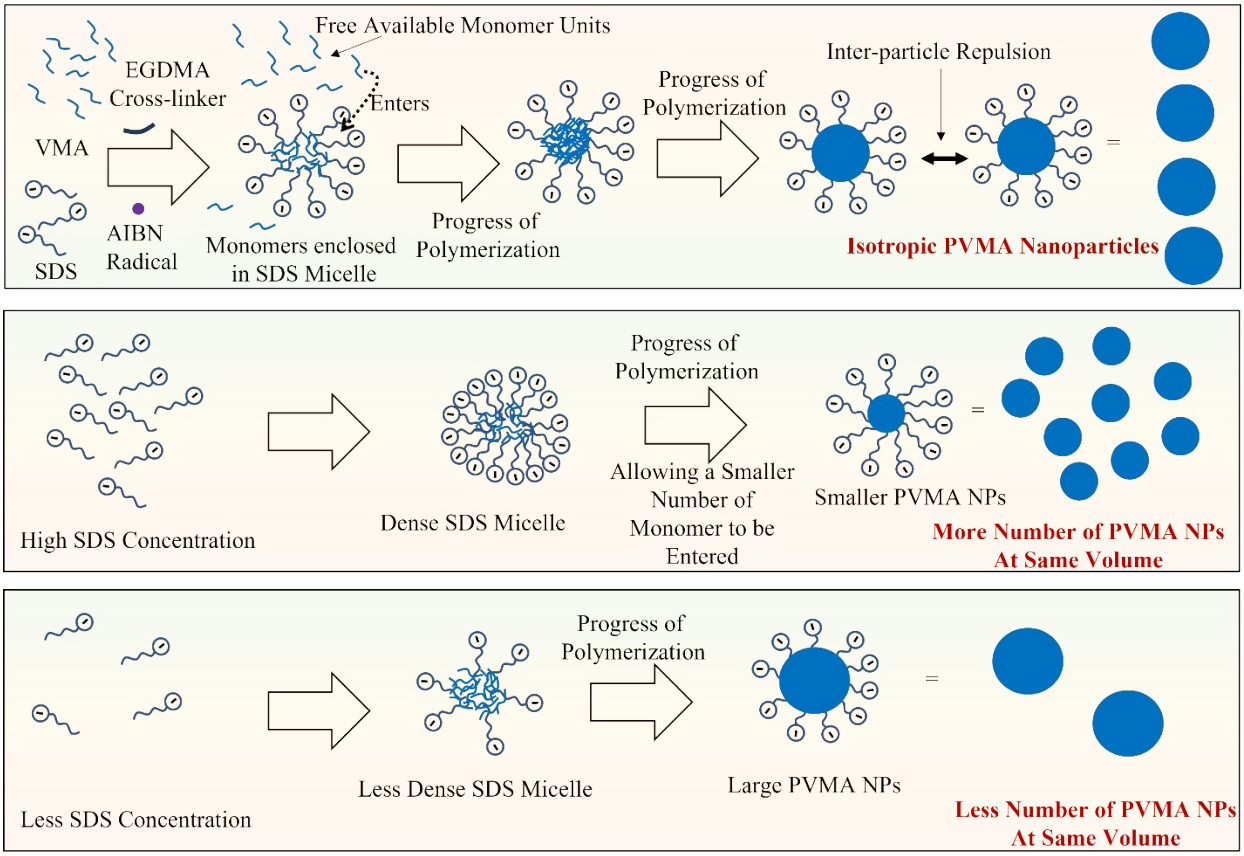
The Zeta potential of the synthesized isotropic PVMA nanoparticles also depends on the concentration of SDS in the aqueous phase. The zeta potential value of the nanoparticles is increased with an increase in the concentration of SDS during the synthesis. The measured zeta potential value of the isotropic nanoparticles is provided in **Supplementary Table S6.1-S6.5.**



Supplementary Figure S1.1. Reaction arrangement for the synthesis of isotropic PVMA nanoparticles by using SDS in the aqueous phase. The impact of five different concentration of SDS (1000 µM, 100 µM, 10 µM, 1 µM, and 0.1 µM) on the formation of PVMA nanoparticles have been investigated during different experiments.

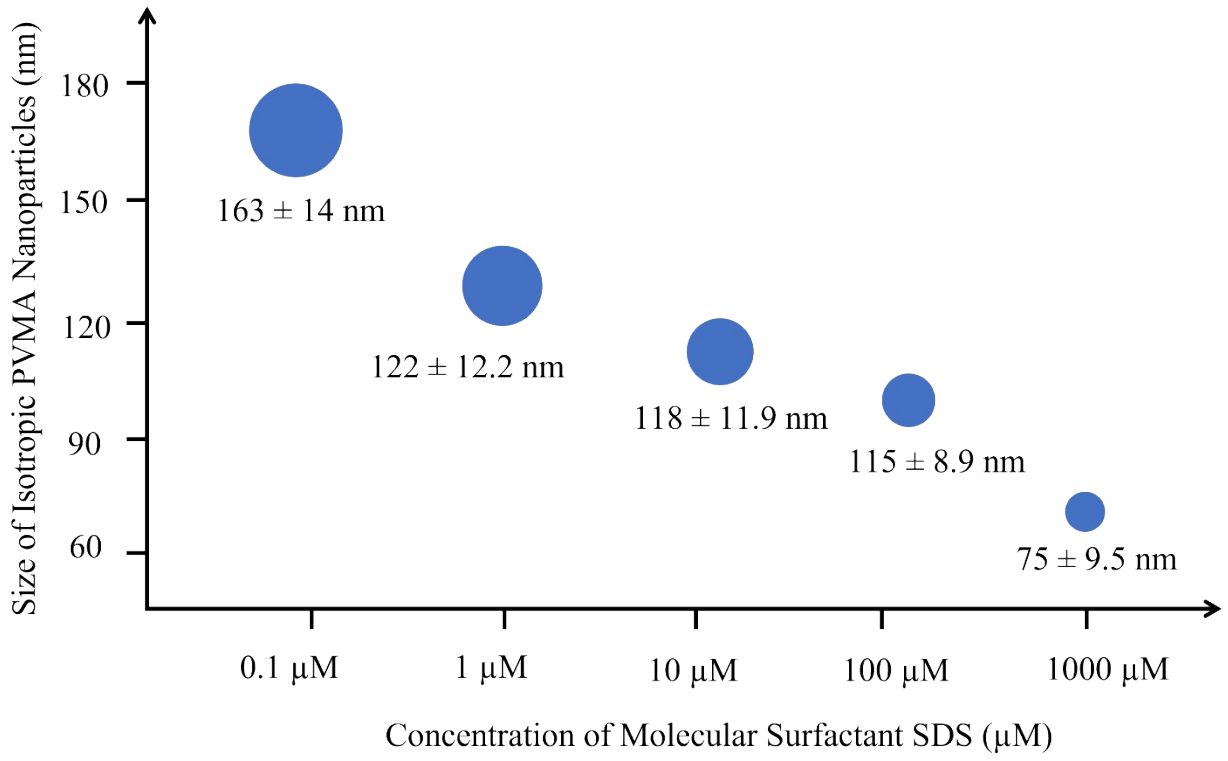
The emulsification of both immiscible liquid phases takes place in the microreactor. Once the emulsion solution is formed, the polymerization is started when the emulsion solution enters the vial which is arranged at the heating block (95 °C) as shown in **Supplementary Figure S1.1**.

Thermal initiator AIBN forms radicals when comes in contact with heating temperature. Radical can immediately attack the polymerization actives sites of the VMA monomers to begin the formation of the PVMA polymer network. The growing long-chain of PVMA can be cross-linked by polymerization of cross-linking agent EGDMA. During the polymerization process, SDS plays a crucial role in controlling the spherical shape, tunable size, and growth of the nanoparticles. A basic depiction of the polymerization process of the isotropic (spherical) PVMA nanoparticles is shown in **Supplementary Figure S1.2**.



Supplementary Figure S1.2. The general proposition of the formation mechanism for the isotropic PVMA nanoparticles in presence of molecular surfactant SDS in the aqueous phase.

As explained above the size of the PVMA nanoparticles are dependent on the concentration of SDS in the aquesou phase. The exact size together with their standard deviation is provided in graphical schematic in **Supplementary Figure S1.3.**

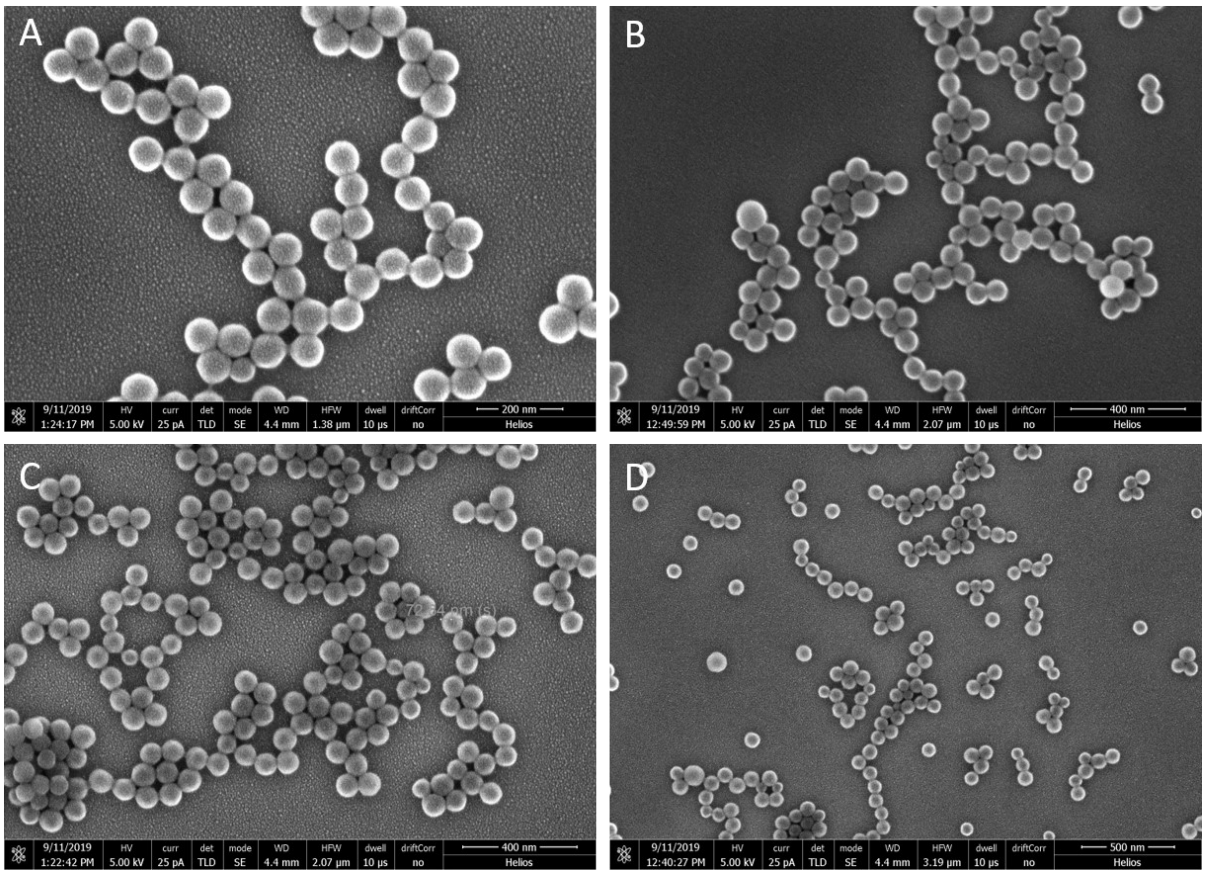


Supplementary Figure S1.3. The general proposition of the formation mechanism for the isotropic PVMA nanoparticles in presence of molecular surfactant SDS in the aqueous phase.

The detailed reaction conditions, zeta potential values, and SEM images of the obtained PVMA nanoparticles are shown in **Supplementary Table S1.1-S1.5** and **Supplementary Figure S1.4-S1.8** below**.**

Supplementary Table S1.1. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S1.4** below.

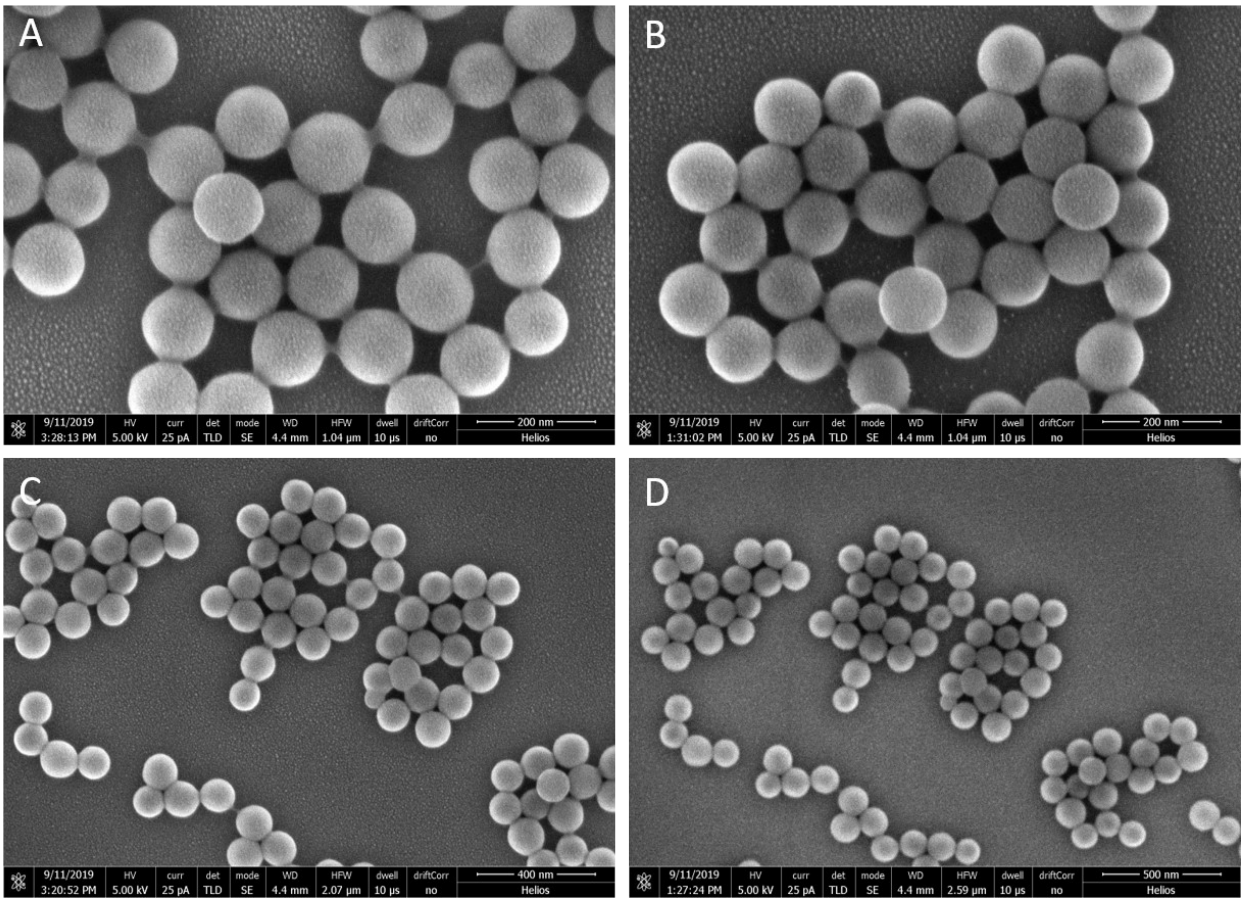
Aqueous Phase	1000 μM SDS
Monomer Phase	990 μL Vinyl methacrylate (VMA) 10 μL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (μL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-36.6 ± 5.12



Supplementary Figure S1.4. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition that given in **Supplementary Table S1.1** above.

Supplementary Table S1.2. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S1.5** below.

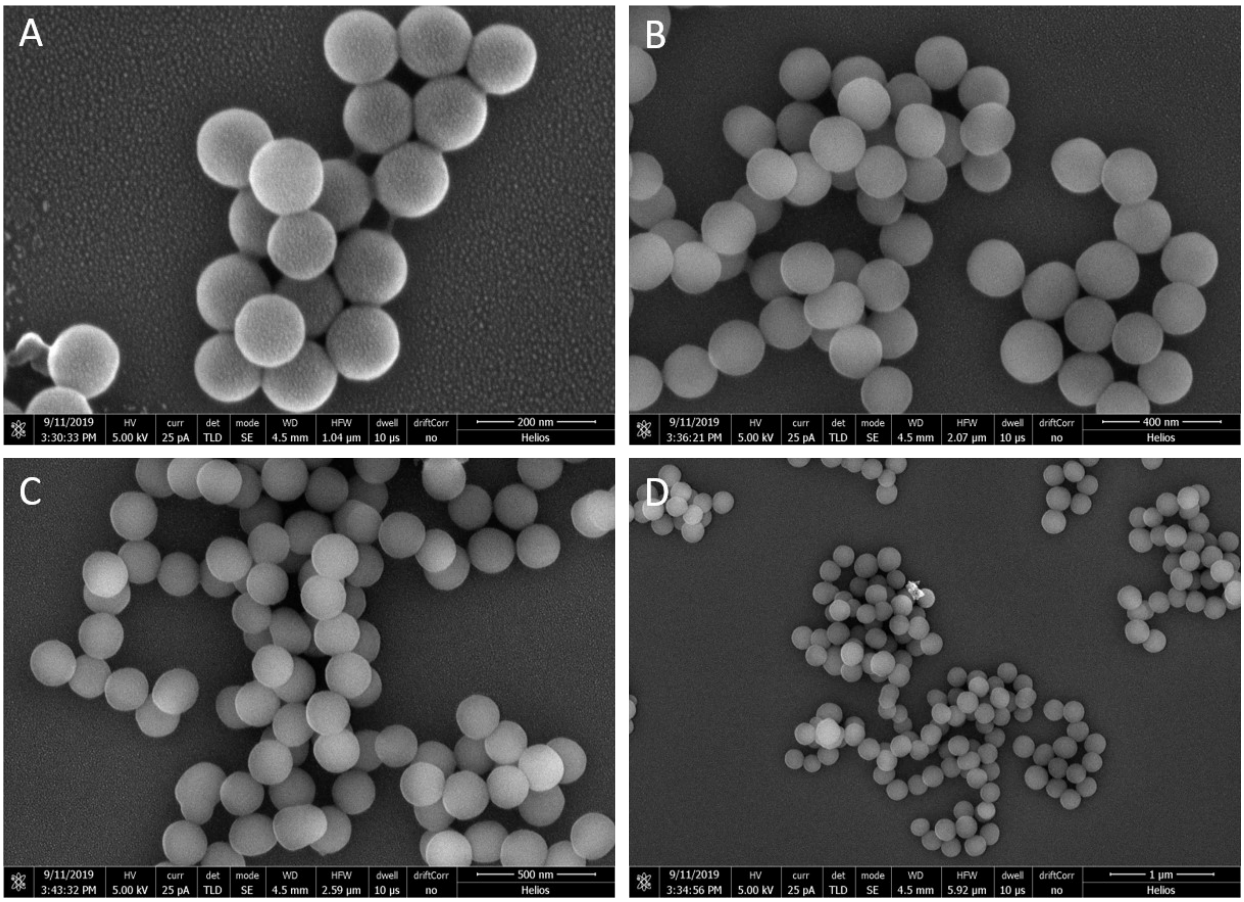
Aqueous Phase	100 µM SDS
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-31.4 ± 6.8



Supplementary Figure S1.5. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition that provided in **Supplementary Table S1.2** above.

Supplementary Table S1.3. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S1.6** below.

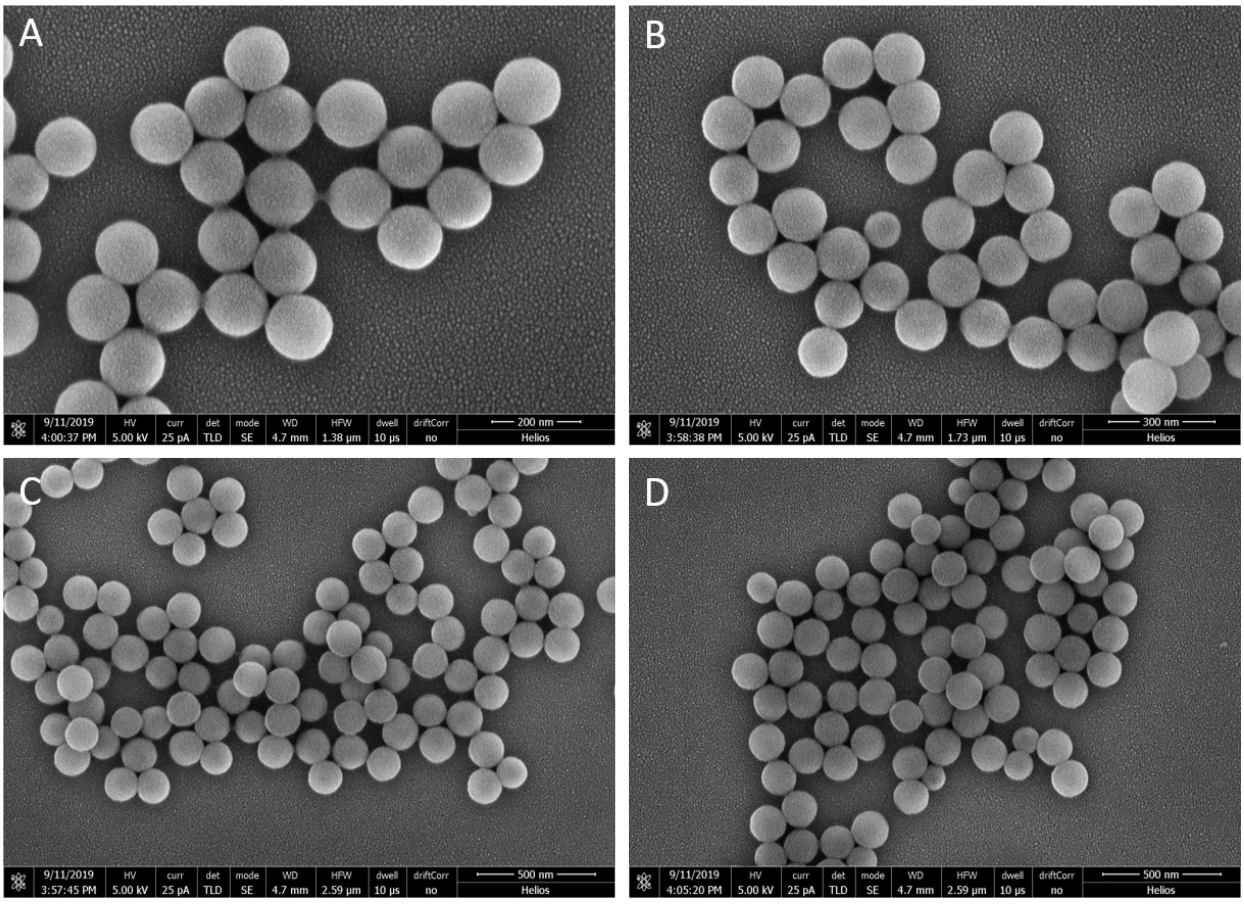
Aqueous Phase	10 μM SDS
Monomer Phase	990 μL Vinyl methacrylate (VMA) 10 μL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (μL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-18.4 ± 3.38



Supplementary Figure S1.6. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition that provided in **Supplementary Table S1.3** above.

Supplementary Table S1.4. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S1.7** below.

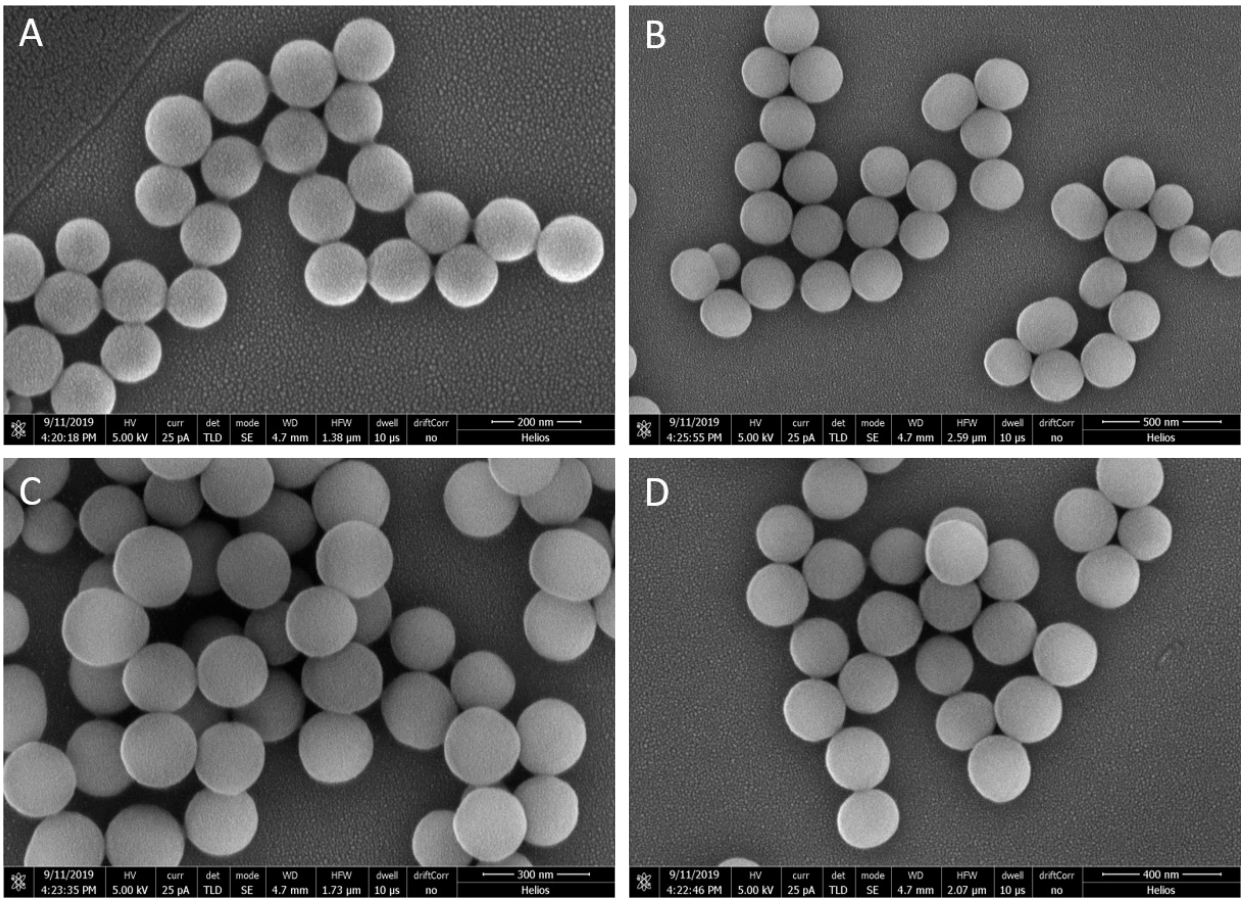
Aqueous Phase	1 μM SDS
Monomer Phase	990 μL Vinyl methacrylate (VMA) 10 μL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (μL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-6.3 ± 3.9



Supplementary Figure S1.7. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition that provided in **Supplementary Table S1.4** above.

Supplementary Table S1.5. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S1.8** below.

Aqueous Phase	0.1 μM SDS
Monomer Phase	990 μL Vinyl methacrylate (VMA) 10 μL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (μL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-3.6 ± 3.27

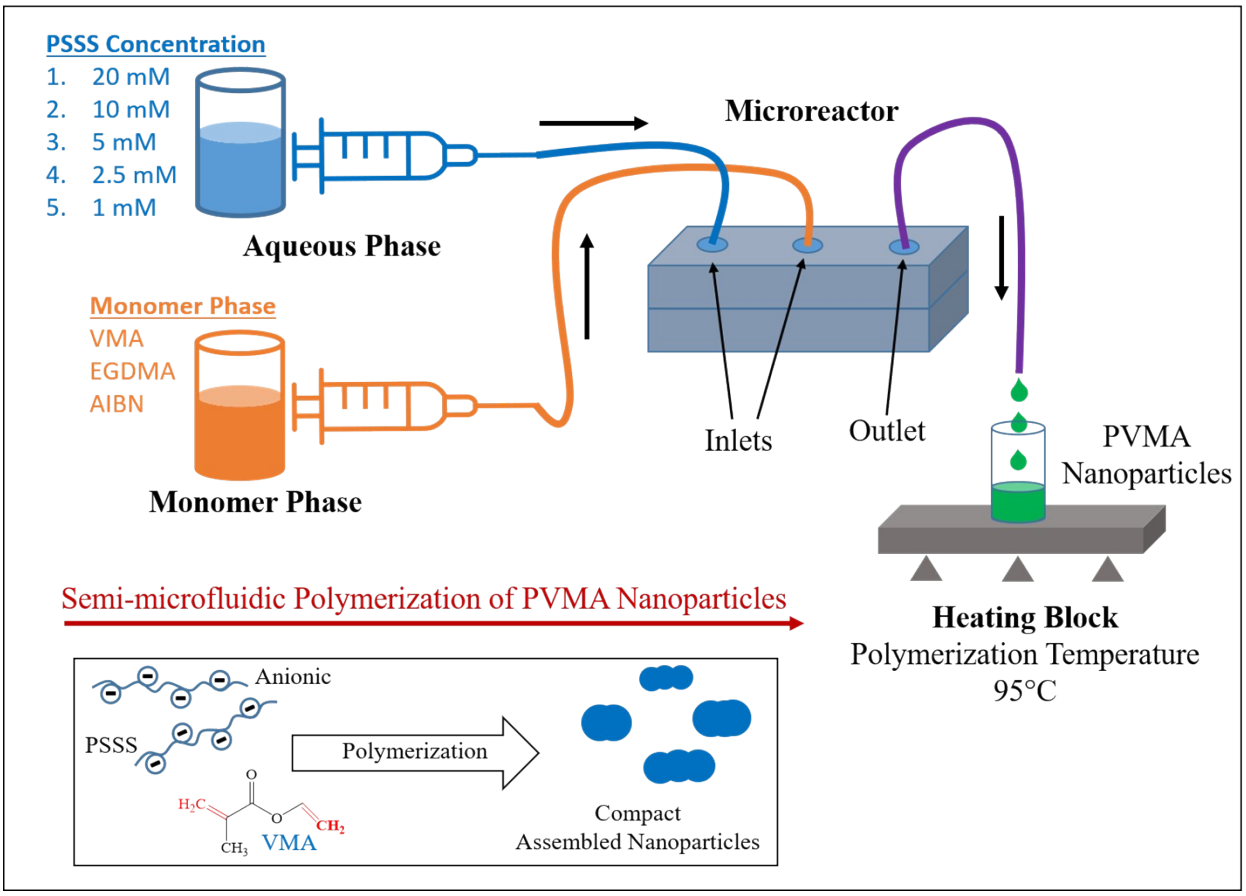


Supplementary Figure S1.8. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition that provided in **Supplementary Table S1.5** above.

2. PVMA Nanoparticles: *Impact of PSSS*

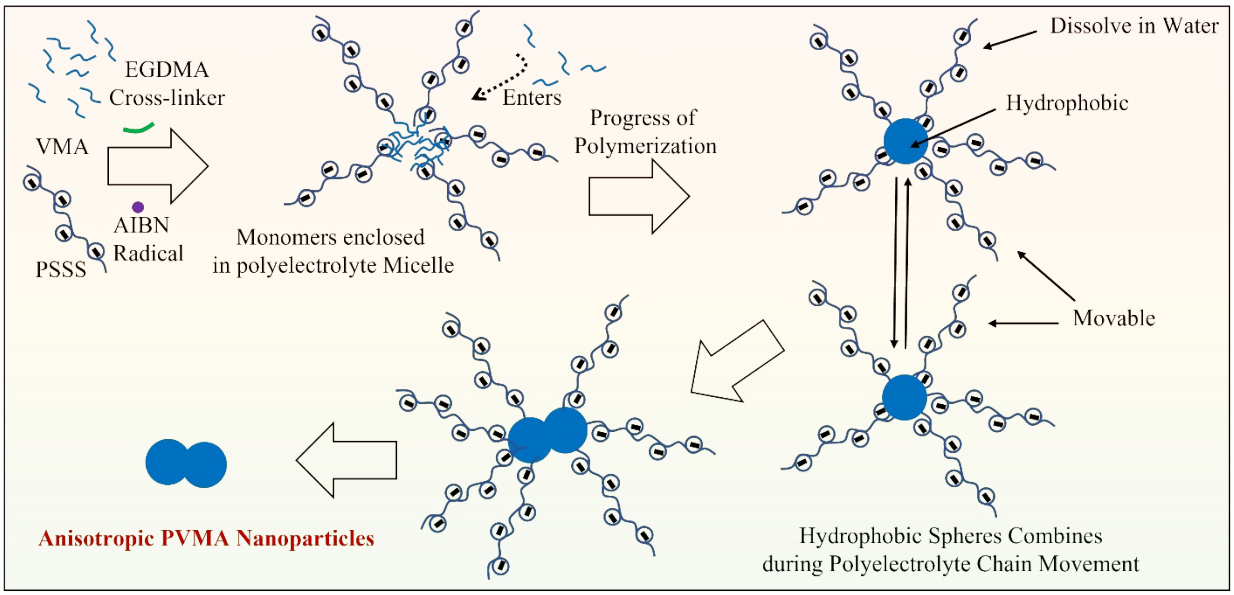
Beyond the isotropic PVMA nanoparticles by molecular surfactant SDS, anisotropic PVMA nanoparticles are the results obtained when interfacial agents with charged polymeric characters were used. Linear type assembling pattern was obtained in the case of using PSSS (anionic polyelectrolytes) in the aqueous phase. PSSS (1,000,000 molecular weight) possess a long chain with multiple anionic charges. It can be imagined that when they are attached to the surface of the nanoparticles during ongoing polymerization, they are flexible and can be moved dynamically. During their movement, there is a possibility where two hydrophobic spheres of smaller size (high energy) assemble and form bigger to minimize the energy (hypothesis). During high PSSS concentration (20 mM, repeating unit concentration), the surface is strongly covered and only one assembling event can be realized (sometimes not at all—which form spheres only) and form compact and sphere-like or ellipsoidal like assemble nanoparticles (assembled from two spheres). With the decrease in the PSSS concentration, assembling patterns in the linear direction can be enhanced and short chain-like PVMA nanoparticles were obtained.

The obtained nanoparticles are anisotropic (non-spherical) in shapes. We extend the concept of polymerization-induced self-assembly during the polymerization process to explain the proposed formation mechanism of linear PVMA nanoparticles. The assembling of the growing spheres can be realized due to the minimization of surface energy to grow bigger. Nanoparticles are hydrophobic. The hydrophilic surface layer is made up of the charged polyelectrolyte PSSS that allow the controlled assembly of PVMA spheres to obtained anisotropic nanoparticles. This process takes place during the ongoing polymerization in a single step, therefore it is termed polymerization-induced self-assembly of PVMA nanoparticles. A basic semi-microfluidic setup for the synthesis of anisotropic PVMA nanoparticles in the presence of PSSS in the aqueous phase is shown in **Supplementary Figure S2.1**.

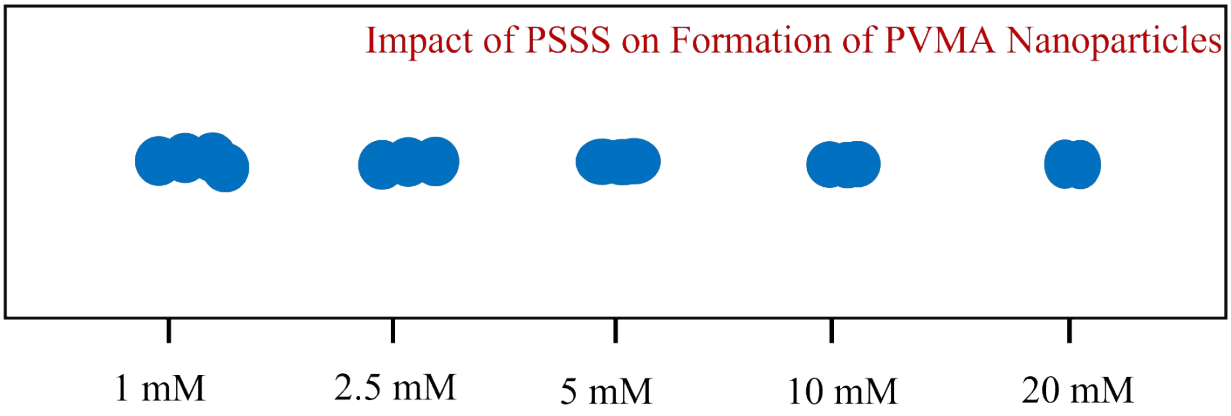


Supplementary Figure S2.1. Reaction arrangement for the synthesis of PVMA nanoparticles by using PSSS in the aqueous phase. The effect of five different concentrations of PSSS (20 mM, 10 mM, 5 mM, 2.5 mM, and 1 mM—Repeating Unit Concentration) on the formation of PVMA nanoparticles have been investigated during different experiments.

In **Supplementary Figure S2.2**, a hypothesis of the formation mechanism for the generation of anisotropic PVMA nanoparticles in presence of PSSS in the aqueous phase is shown in the cartoon form. Moreover, the obtained shape of the PVMA nanoparticles at five different concentrations of PSSS is shown in **Supplementary Figure S2.3** below.



Supplementary Figure S2.2. Proposed formation mechanism of anisotropic (non-spherical) PVMA nanoparticles during the application of PSSS in the aqueous phase.

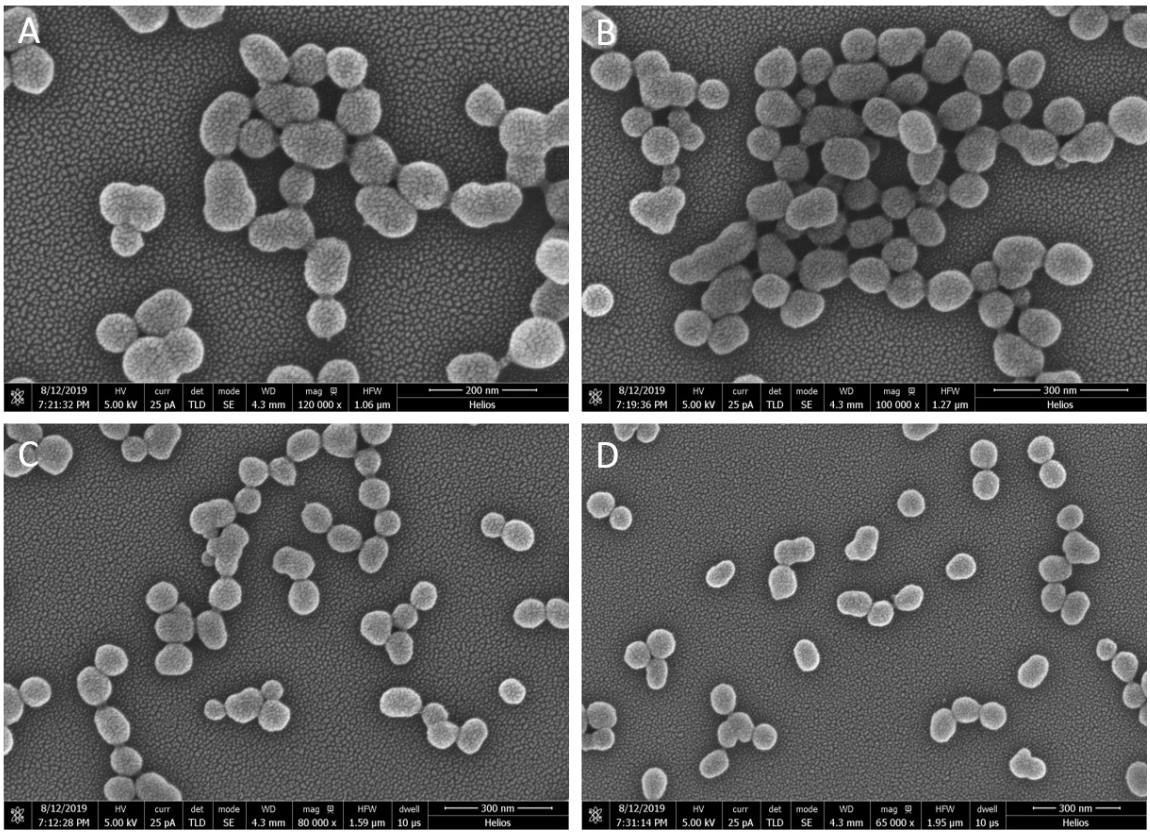


Supplementary Figure S2.3. Cartoons of the polymer nanoparticles were obtained at different PSSS concentrations in the aqueous phase during the polymerization process.

The detailed reaction conditions, zeta potential values, and SEM images of the obtained PVMA nanoparticles in the case of using PSSS as an interfacial agent in the aquesou phase during polymerization process are shown in **Supplementary Table S2.1-S2.5** and **Supplementary Figure S2.4-S2.8** below**.**

Supplementary Table S2.1. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S2.4** below.

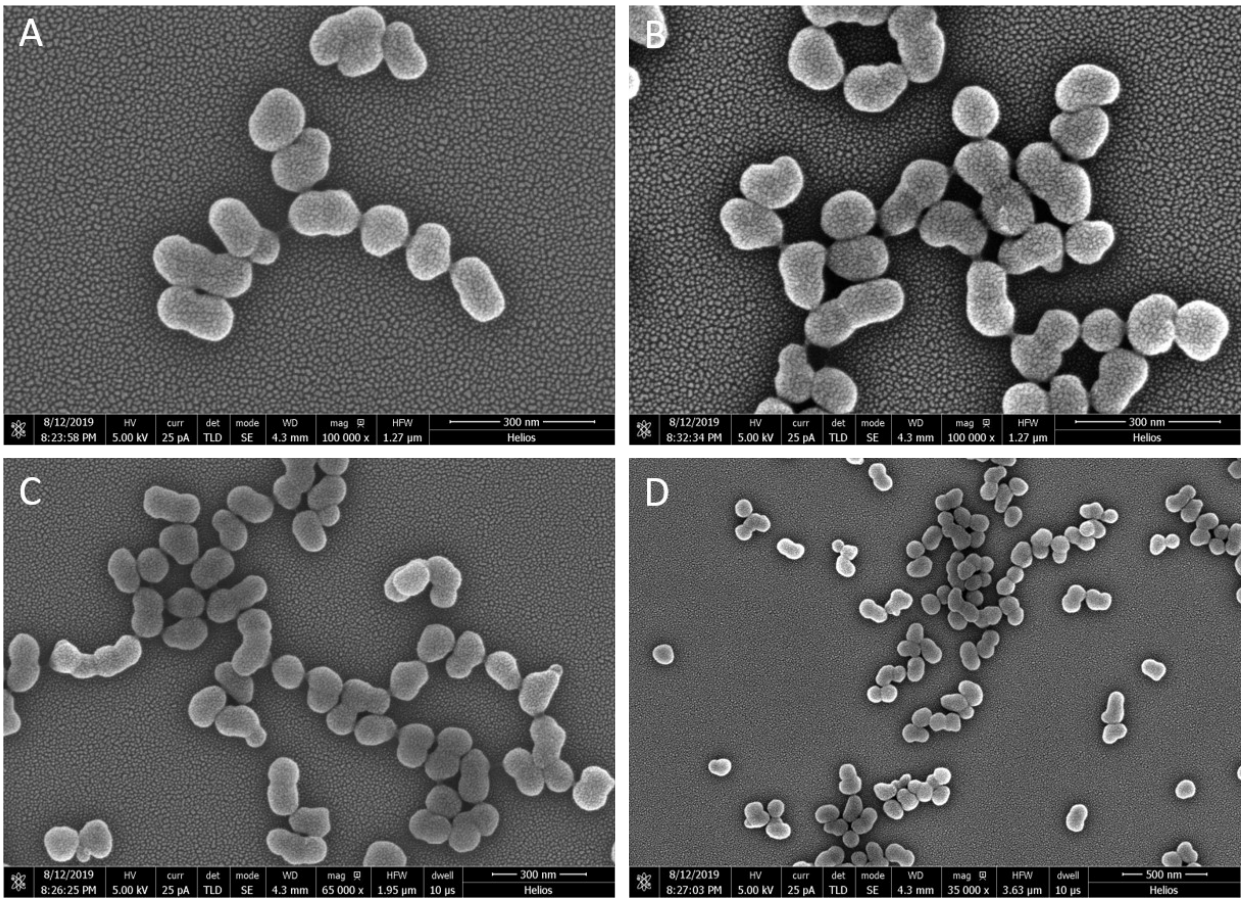
Aqueous Phase	20 mM PSSS (1,000,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-47.3 ± 4.03



Supplementary Figure S2.4. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S2.1** above.

Supplementary Table S2.2. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S2.5** below.

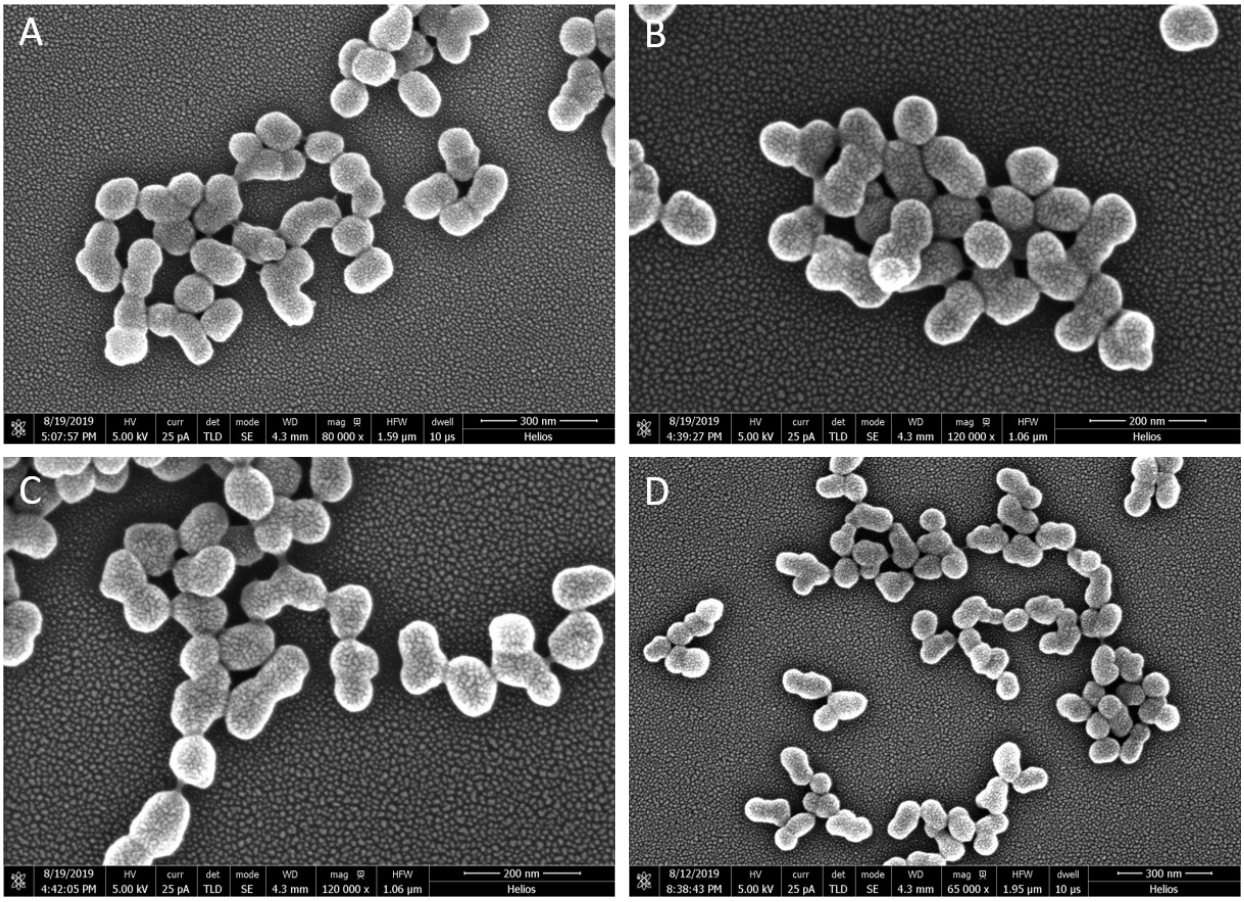
Aqueous Phase	10 mM PSSS (1,000,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-45.8 ± 4.26



Supplementary Figure S2.5. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition are provided in **Supplementary Table S2.2** above.

Supplementary Table S2.3. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S2.6** below.

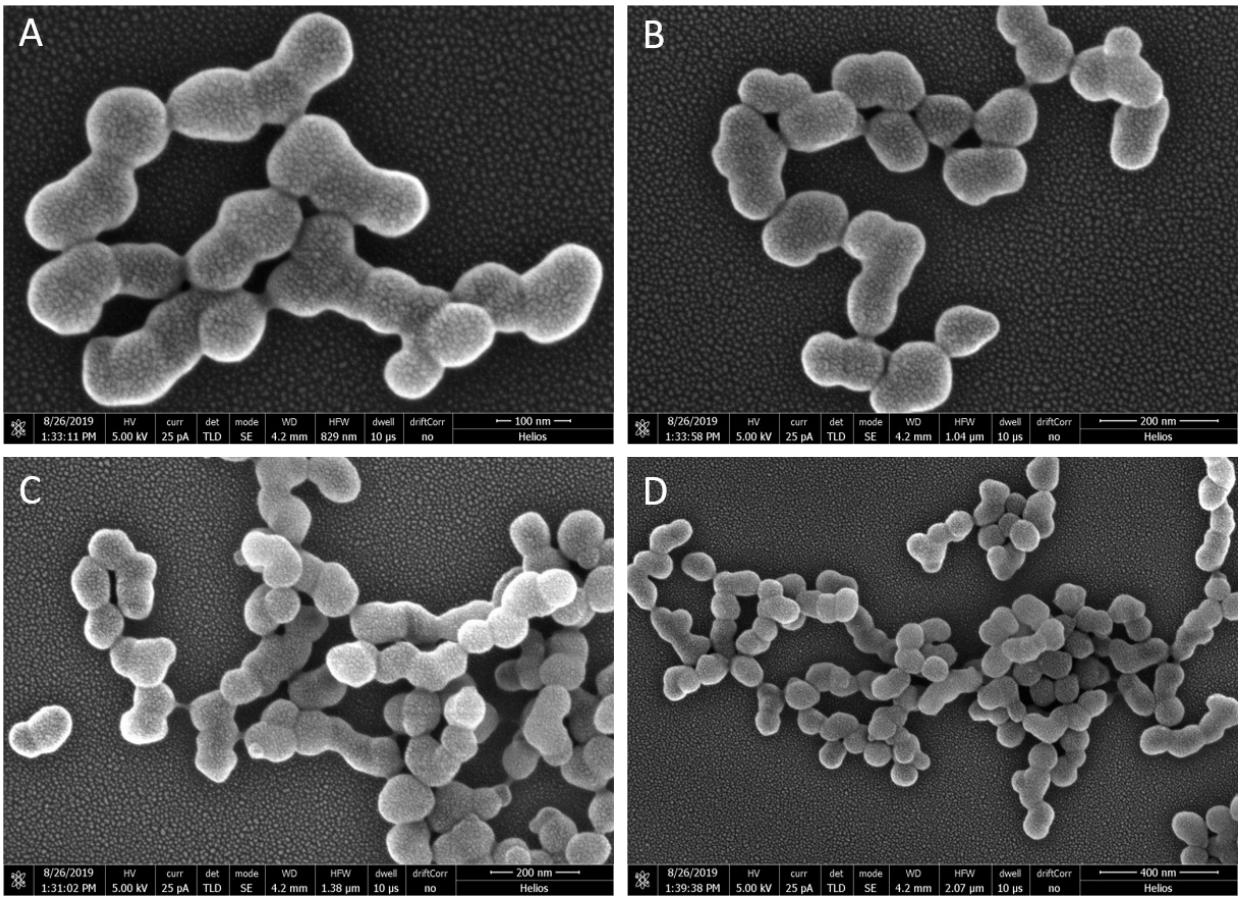
Aqueous Phase	5 mM PSSS (1,000,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-40.5 ± 5.79



Supplementary Figure S2.6. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S2.3** above.

Supplementary Table S2.4. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S2.7** below.

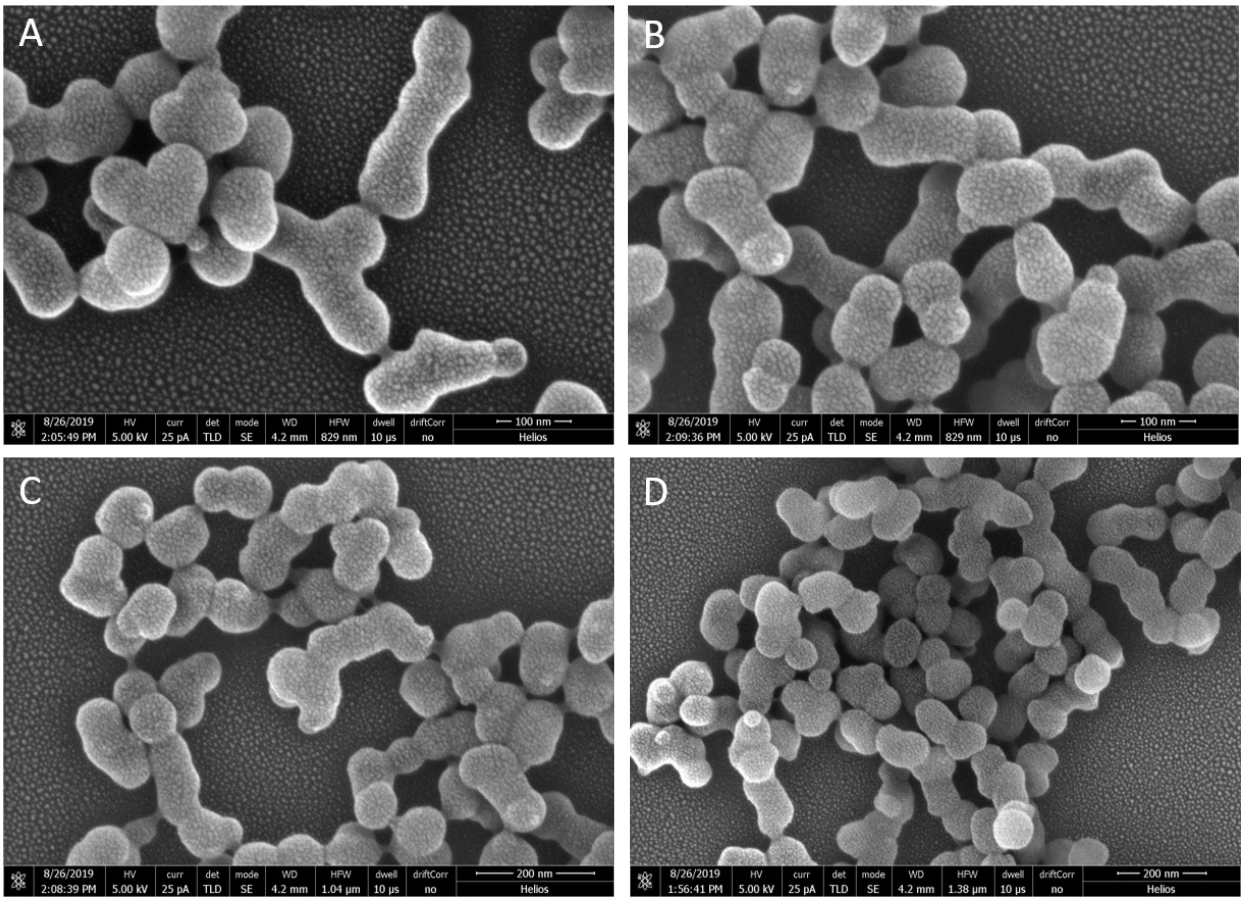
Aqueous Phase	2.5 mM PSSS (1,000,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-38.8 ± 4.83



Supplementary Figure S2.7. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S2.4** above.

Supplementary Table S2.5. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S2.8** below.

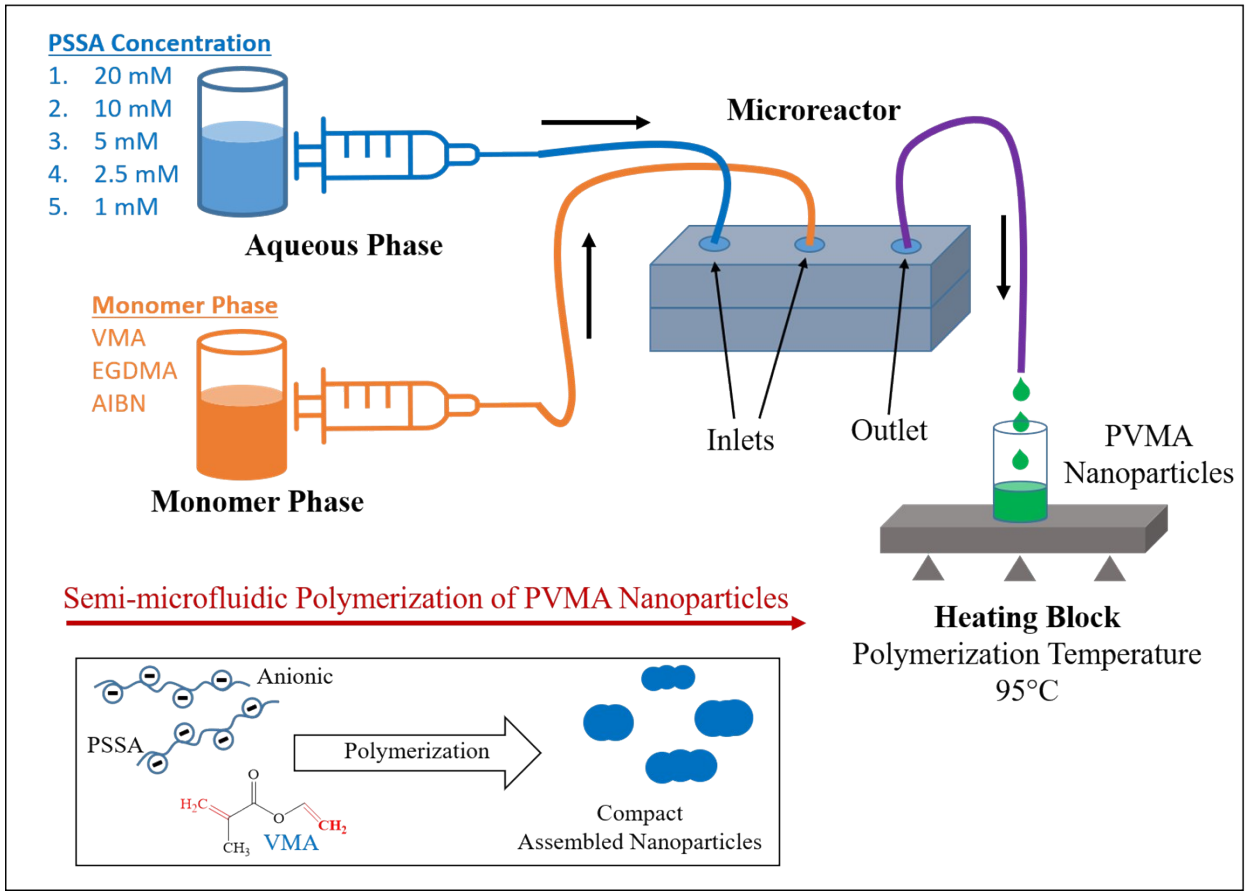
Aqueous Phase	1 mM PSSS (1,000,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-37 ± 3.9



Supplementary Figure S2.8. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S2.5** above.

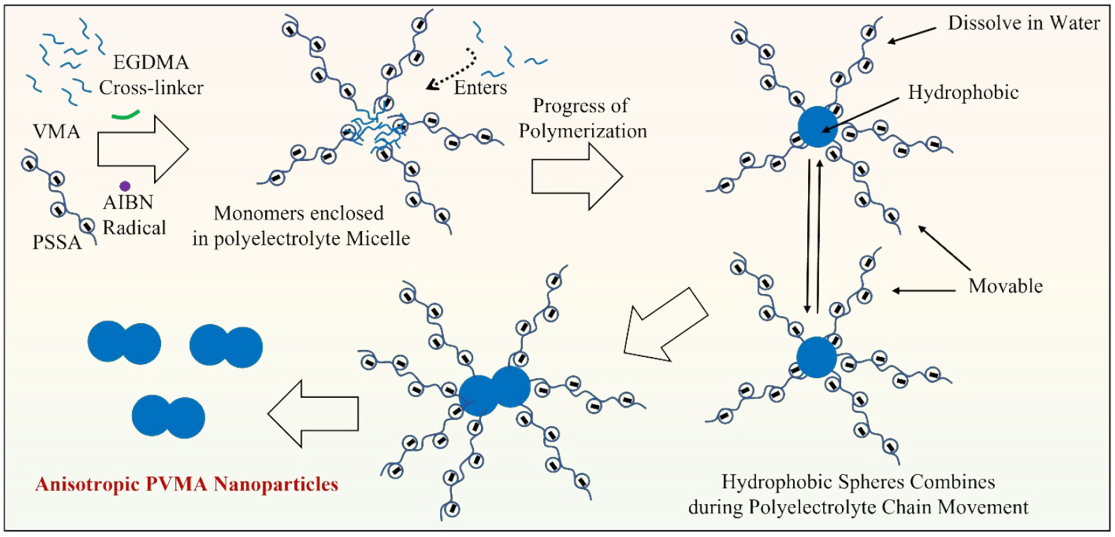
3. PVMA Nanoparticles: *Impact of PSSA*

Anisotropic PVMA nanoparticles during the application of anionic polyelectrolyte PSSA are obtained similarly to the case of a PSSS interfacial agent. The formation mechanism of the PVMA nanoparticles by PSSA can be explained based on the polymerization-induced self-assembly concept. To investigate the impact of PSSA on the formation of PVMA nanoparticles, we have used seven different repeating unit concentrations of PSSS in the aqueous phase: 20 mM, 10 mM, 5 mM, 2.5 mM, 1 mM, 0.5 mM, and 0.1 mM. The polymerization reaction was performed at 95°C. the initial emulsion has been formed in the microreactor, and completion of the polymerization process takes place outside of the microreactor at a heating block. Overall, the full reaction is a semi-microfluidic single-step polymerization process *via* polymerization-induced self-assembly to form the anisotropic PVMA nanoparticles. A basic reaction setup for the PVMA nanoparticles during the application of PSSA is shown in **Supplementary Figure S3.1**.

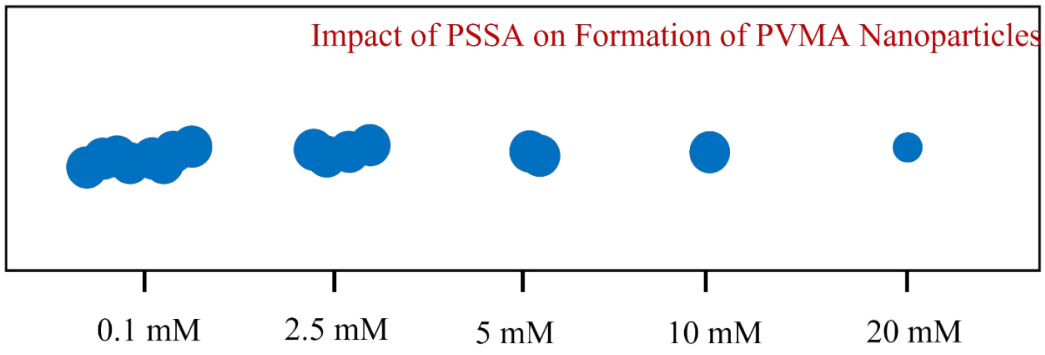


Supplementary Figure S3.1. Reaction arrangement for the synthesis of PVMA nanoparticles by using PSSA in the aqueous phase. The effect of five different concentrations of PSSA (20 mM, 10 mM, 5 mM, 2.5 mM, and 1 mM—Repeating Unit Concentration) on the formation of PVMA nanoparticles have been investigated during different experiments.

A proposed formation mechanism for the anisotropic PVAM nanoparticles in presence of PSSA in the aqueous phase is shown in **Supplementary Figure S3.2**. Moreover, the obtained shapes of the PVMA nanoparticles at different concentration of PSSA is shown in **Supplementary Figure S3.3**.



Supplementary Figure S3.2. Proposed formation mechanism of anisotropic (non-spherical) PVMA nanoparticles during the application of PSSA in the aqueous phase.

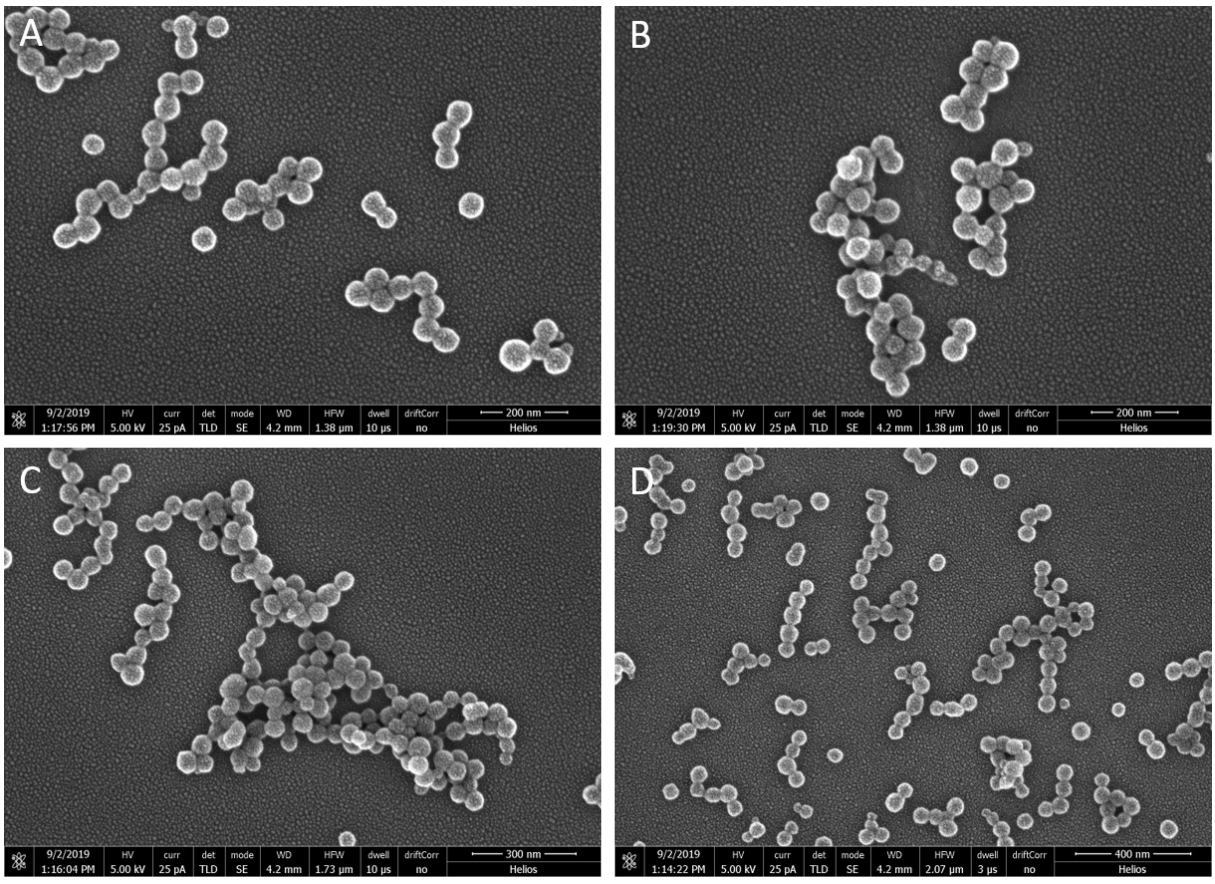


Supplementary Figure S3.3. Cartoons of the polymer nanoparticles were obtained at different PSSA concentrations in the aqueous phase during the polymerization process.

The detailed reaction conditions, zeta potential values, and SEM images of the obtained PVMA nanoparticles during application of PSSA in aquesou phase are shown in **Supplementary Table S3.1-S3.7** and **Supplementary Figure S3.4-S3.9** below**.**

Supplementary Table S3.1. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S3.4** below.

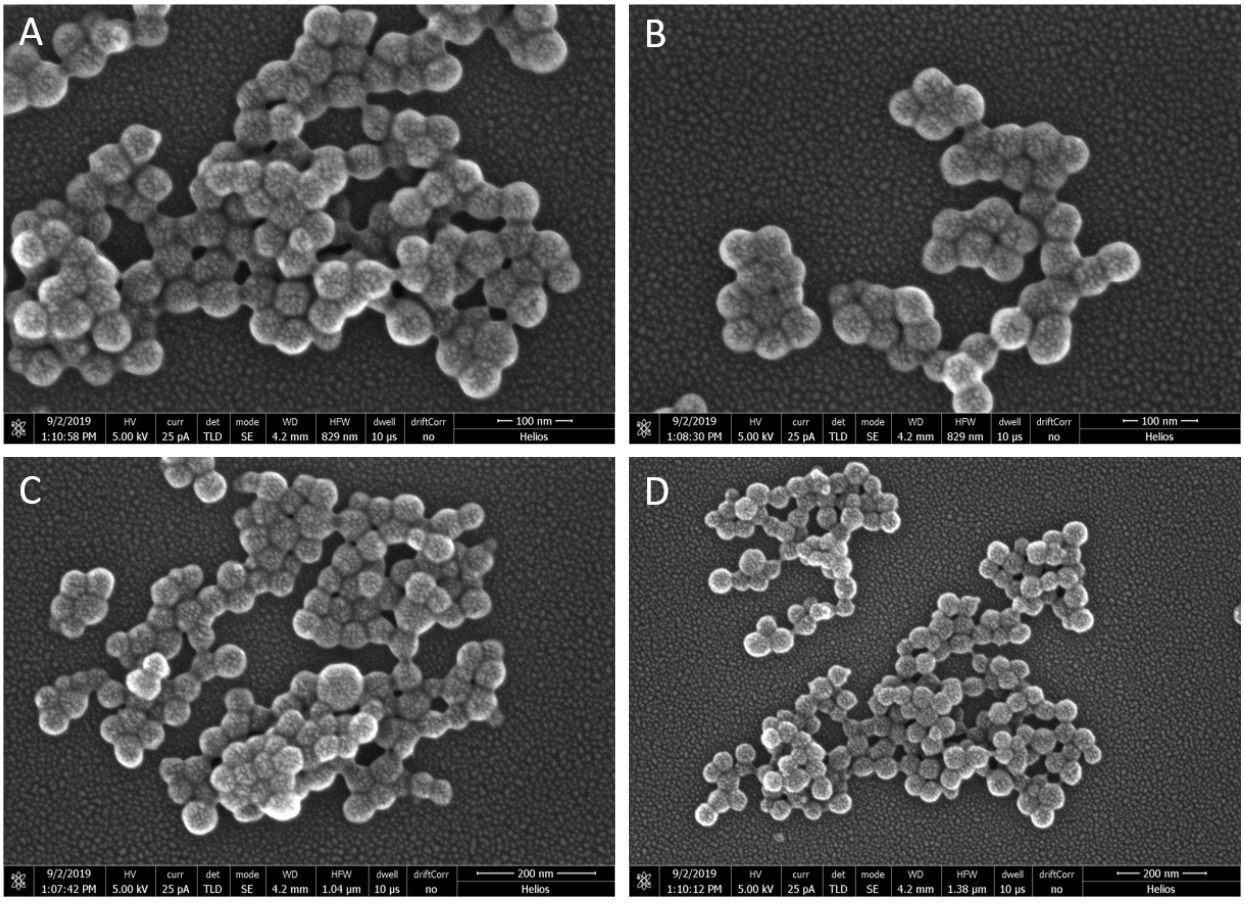
Aqueous Phase	20 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-50.3 ± 4.52



Supplementary Figure S3.4. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S3.1** above.

Supplementary Table S3.2. Reaction condition, key responsibl2e reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S3.5** below.

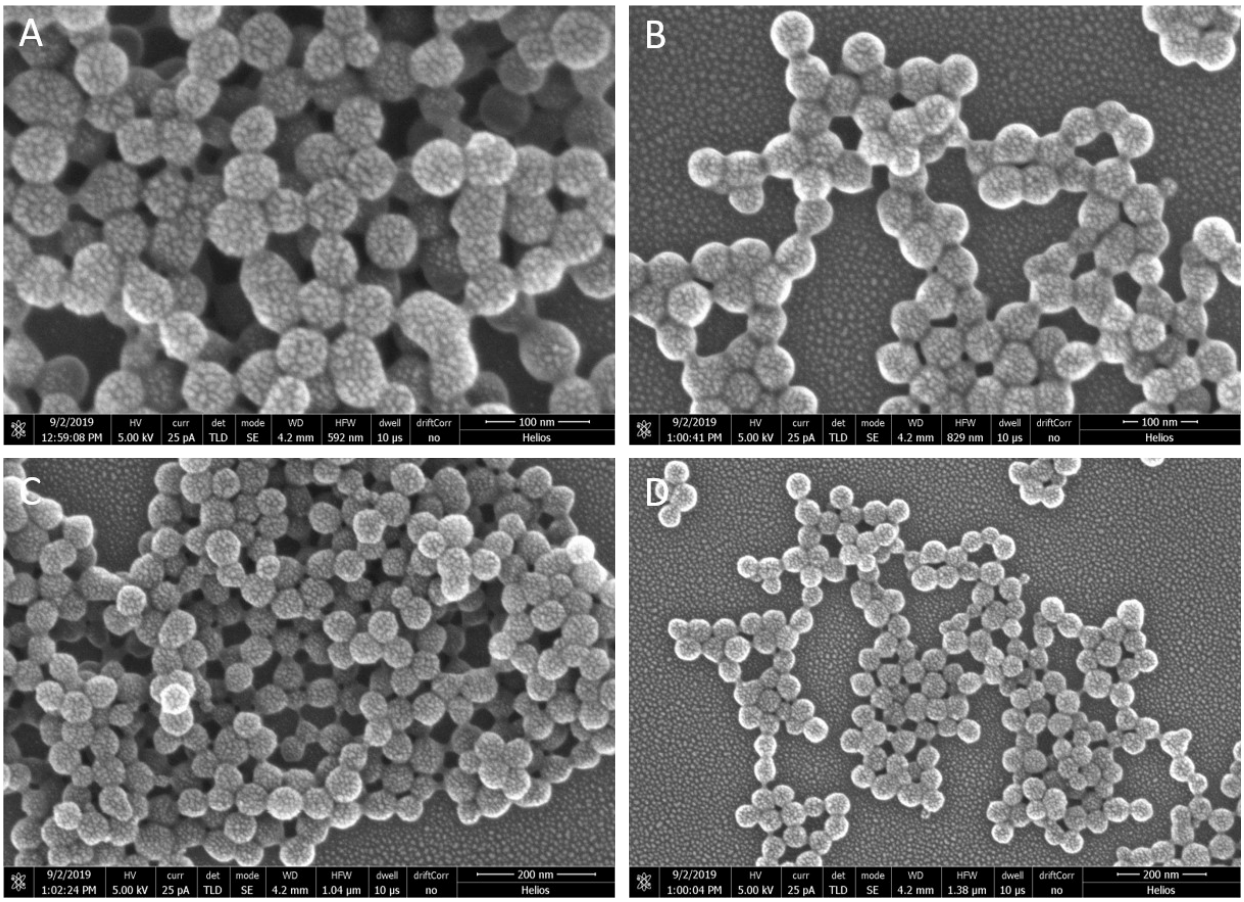
Aqueous Phase	10 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-56.3 ± 3.58



Supplementary Figure S3.5. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S3.2** above.

Supplementary Table S3.3. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S3.6** below.

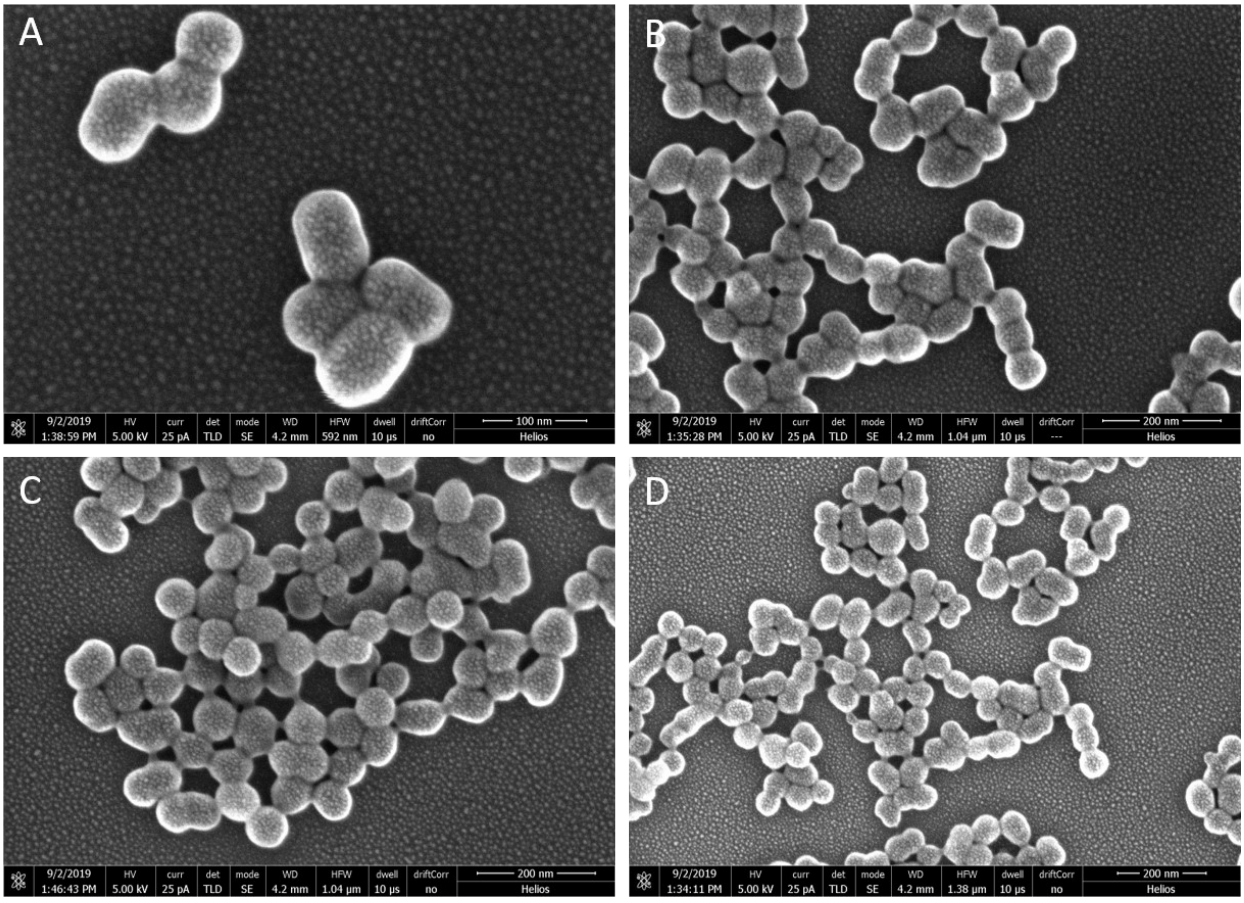
Aqueous Phase	5 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-38.1 ± 5.62



Supplementary Figure S3.6. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S3.3** above.

Supplementary Table S3.4. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S3.7** below.

Aqueous Phase	2.5 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-61.1 ± 4.9



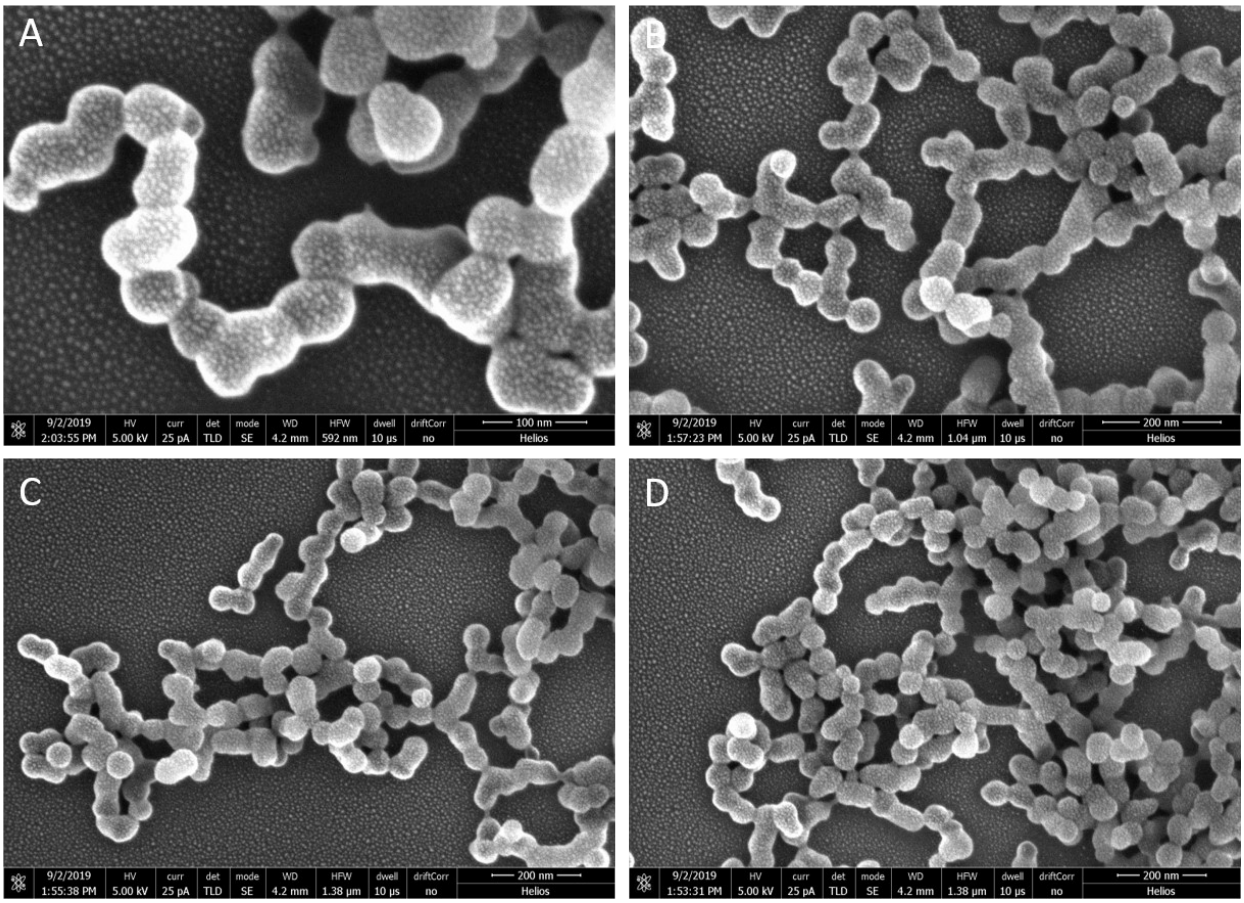
Supplementary Figure S3.7. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S3.4** above.

Supplementary Table S3.5. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table.

Aqueous Phase	1 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-46.6 ± 4.19

Supplementary Table S3.6. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S3.8** below.

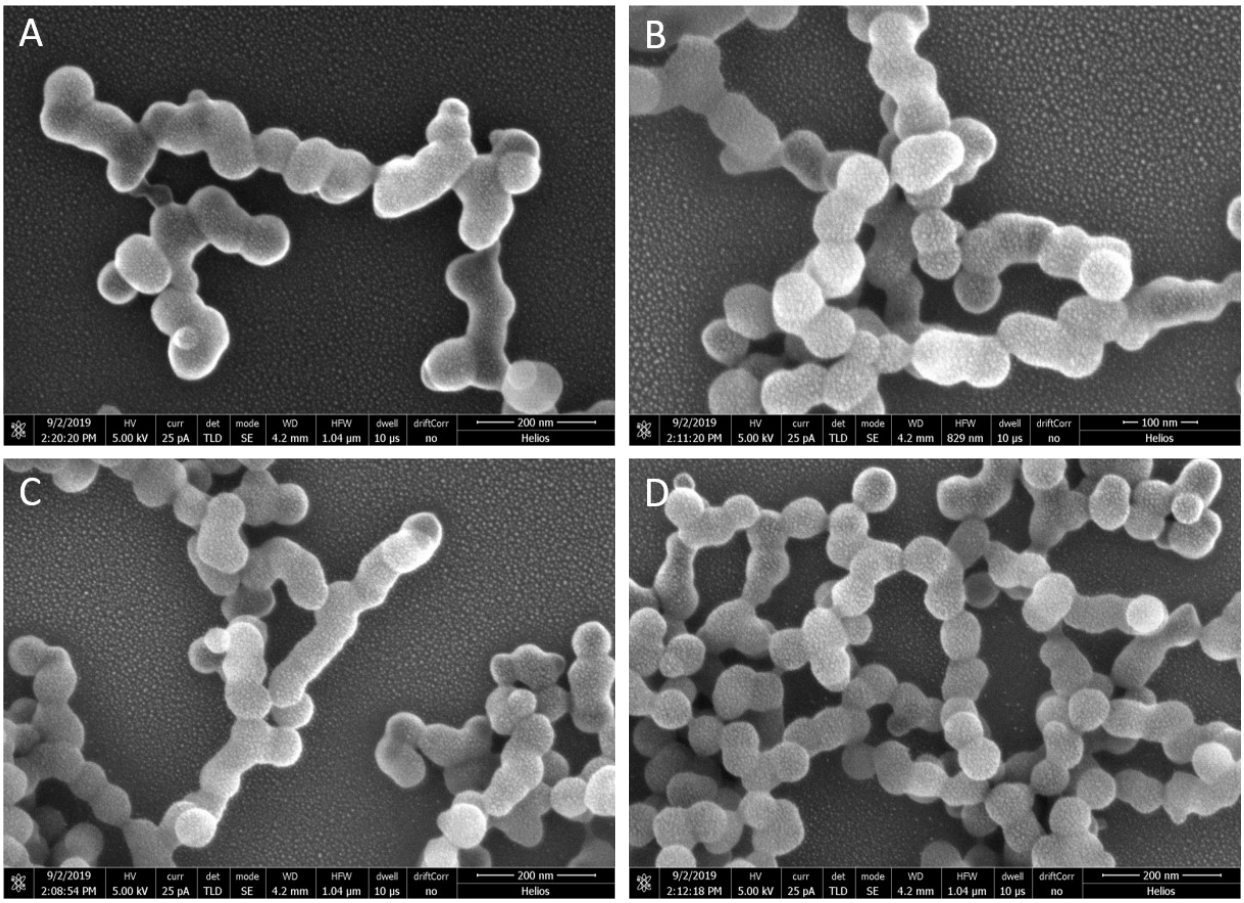
Aqueous Phase	0.5 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-28.6 ± 4.07



Supplementary Figure S3.8. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S3.6** above.

Supplementary Table S3.7. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S3.9** below.

Aqueous Phase	0.1 mM PSSA (200,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-31.1 ± 3.84

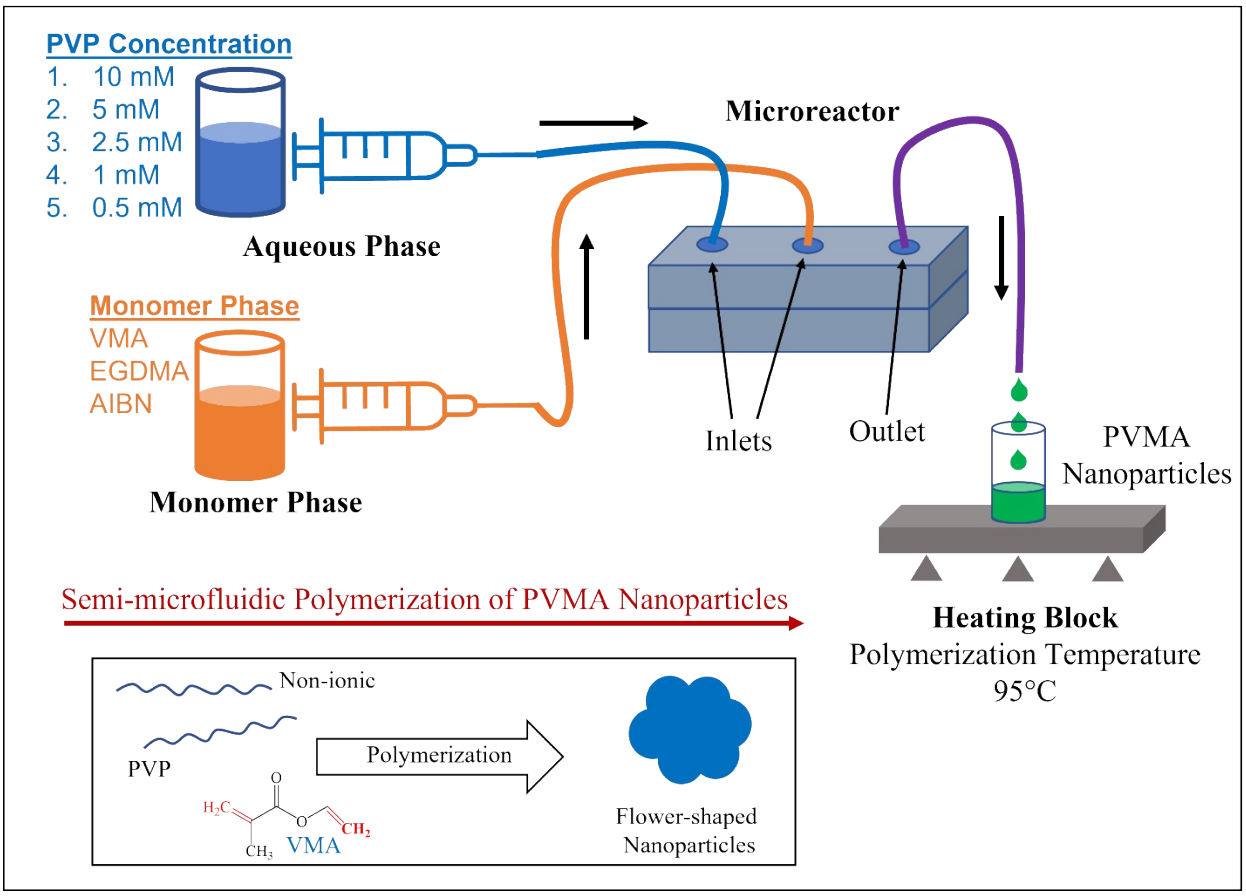


Supplementary Figure S3.9. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S3.7** above.

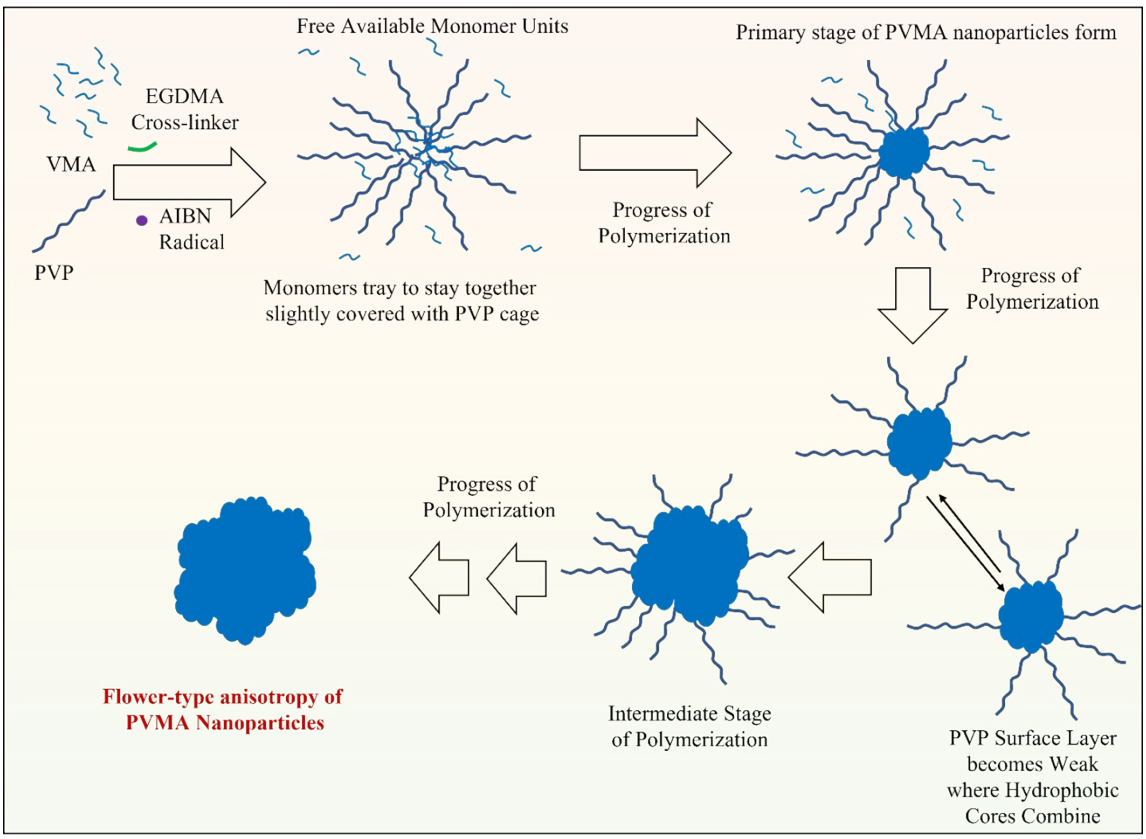
4. PVMA Nanoparticles: *Impact of PVP.*

When non-ionic polymer PVP was used as an interfacial agent in the aqueous phase, a flower-type assembly of the PVMA nanoparticles has been obtained. PVP is solely responsible for allowing controlled assembly during polymerization where growing spheres assemble in a specific manner to form a flower-like assembly of the polymer nanoparticles. In the previous work, this proof-of-concept has been demonstrated in the case of model system polymethyl methacrylate (PMMA) nanoparticles. In the case of PMMA nanoparticles, it was found that the assembling of PMMA nanoparticles during one-step continuous polymerization has been obtained at all concentration range (higher to lower) with slight variation in the mean diameter. That means when a higher concentration of PVP (10 mM) was used, the mean size of the PMMA nanoparticles was small. And, with lowering down of PVP concentration, mean size was increasing. Consequently, larger flower-type assembled PMMA nanoparticles were obtained in case of lower PVP concentration (0.5 mM).

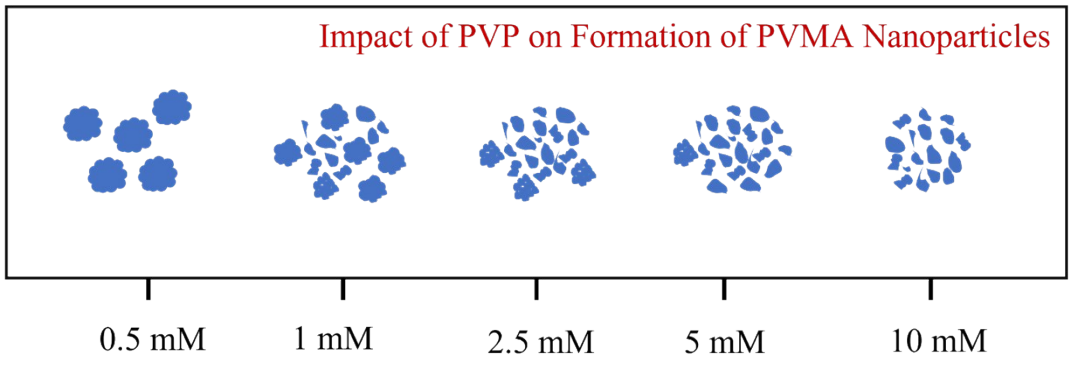
The situation becomes a change in the case of PVMA nanoparticles where the flower-type assembly was realized only at the lower concentration of PVP (0.5 mM, repeating unit concentration). The mechanistic study is yet to perform in future research work, but we believe that such behavior of the assembly in the case of PVMA nanoparticles at the selective concentration of PVP is due to the dual polymerization sites available in the vinyl methacrylate monomers. Moreover, this assembly pattern only at the lower PVP concentration gives the clue of the role of PVP in the formation of controlled assembly in the form of flower-type self-assembled nanoparticles under the shelter of PVP. Also, this study in the case of PVMA nanoparticles has proved that a specific concentration of PVP is required where assembling can take place unlike the situation in the case of PMMA nanoparticles. A basic reaction setup for the formation of anisotropic PVMA nanoparticles during the application of PVP in the aqueous phase is shown in **Supplementary Figure S4.1**. In addition, a schematic of the proposed formation mechanism for the anisotropic PVMA nanoparticles in presence of PVP in the aqueous phase is shown in **Supplementary Figure S4.2**. Moreover, the obtained shapes of the PVMA nanoparticles at different PVP concentration is shown in **Supplementary Figure S4.3**.



Supplementary Figure 4.1. Reaction arrangement for the synthesis of PVMA nanoparticles by using PVP in the aqueous phase. The effect of five different concentrations of PVP (10 mM, 5 mM, 2.5 mM, 1 mM, and 0.5 mM—Repeating Unit Concentration) on the formation of PVMA nanoparticles has been investigated during different experiments.



Supplementary Figure S4.2. Formation mechanism of anisotropic (non-spherical) PVMA nanoparticles during the application of PVP as an interfacial agent in the aqueous phase during the polymerization reaction.

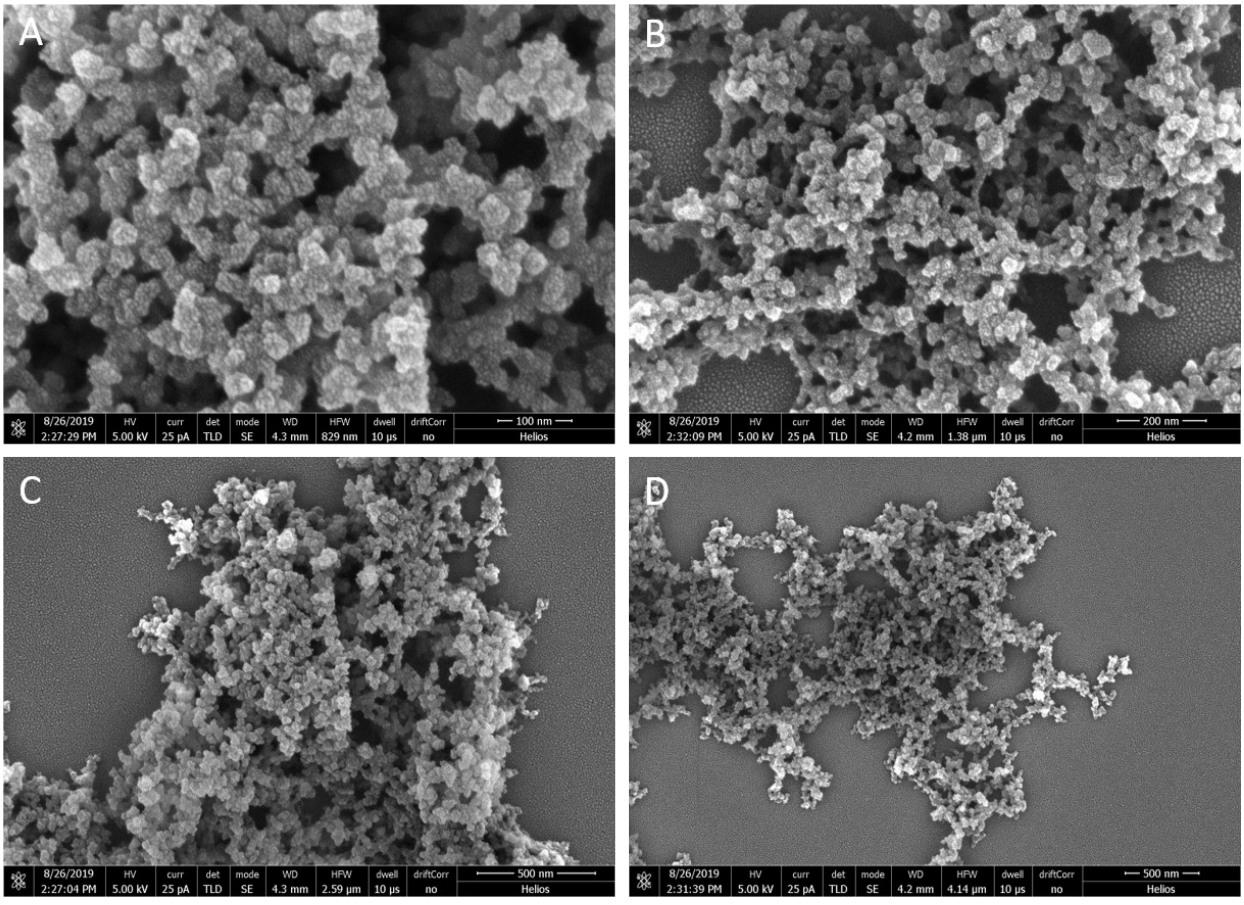


Supplementary Figure S4.3. Cartoons of the polymer nanoparticles were obtained at different PVP concentrations in the aqueous phase during the polymerization process.

The detailed reaction conditions, zeta potential values, and SEM images of the obtained PVMA nanoparticles during application of PVP in aquesou phase are shown in **Supplementary Table S4.1-S4.5** and **Supplementary Figure S4.4-S4.8** below**.**

Supplementary Table S4.1. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S4.4** below.

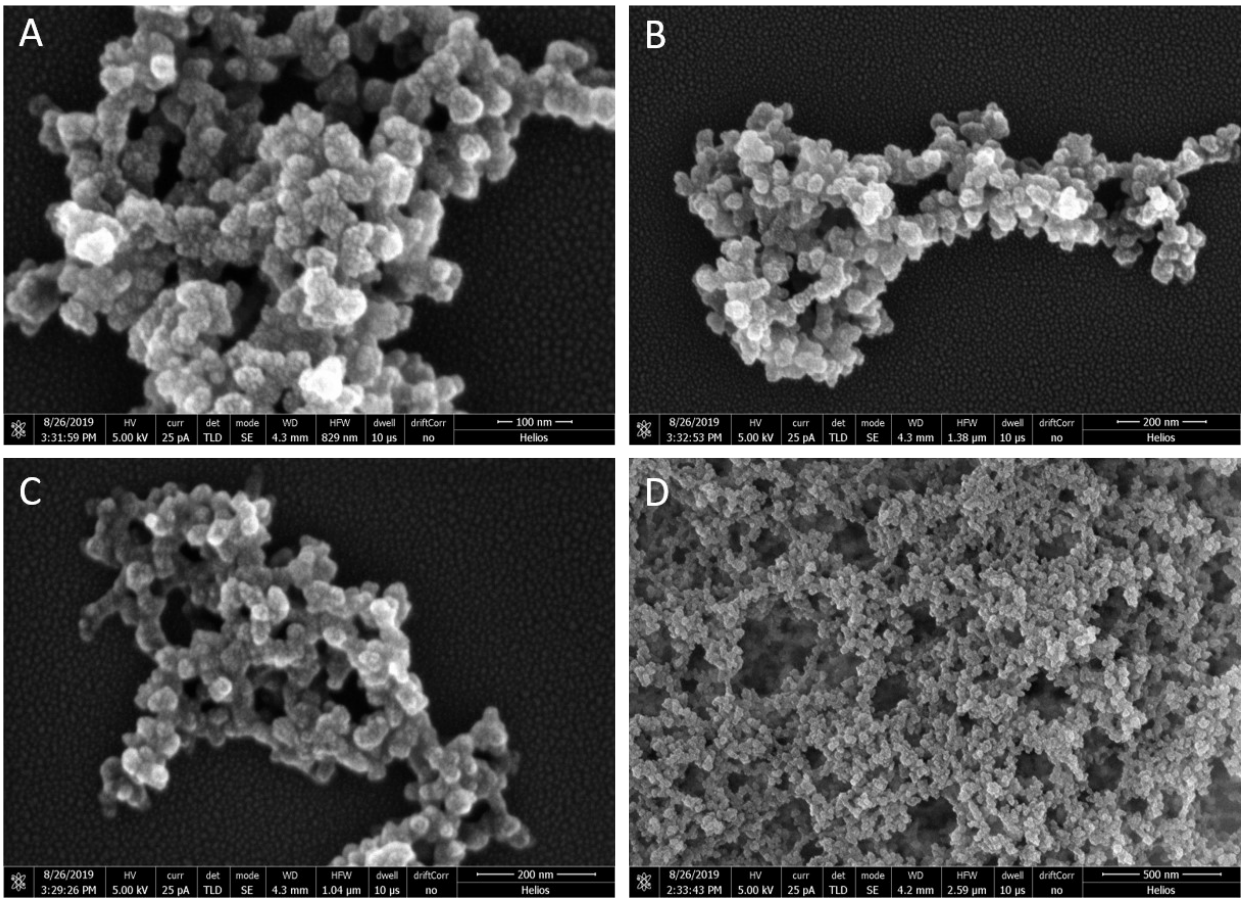
Aqueous Phase	10 mM PVP (55,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-17.3 ± 5.44



Supplementary Figure S4.4. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S4.1** above.

Supplementary Table S4.2. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S4.5** below.

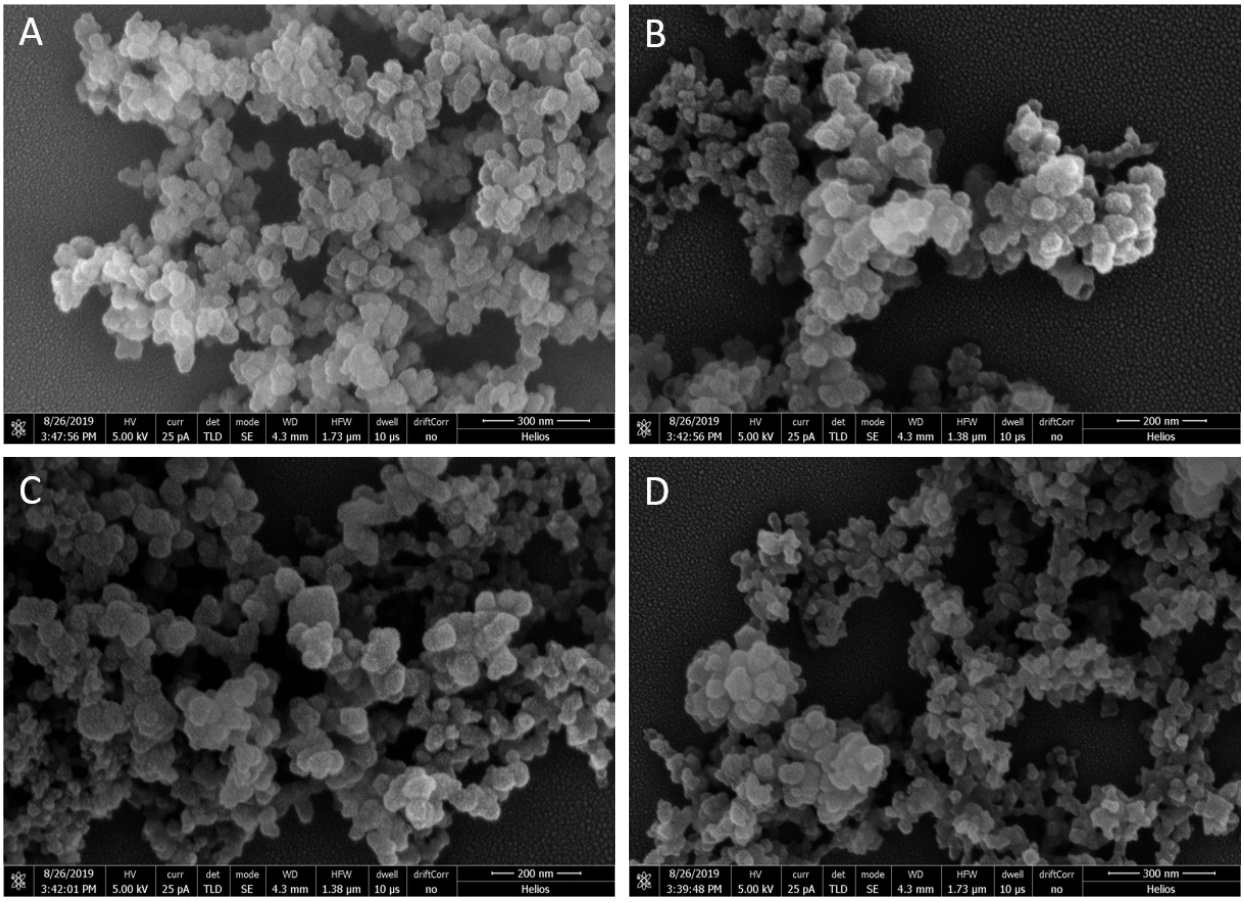
Aqueous Phase	5 mM PVP (55,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-16.7 ± 2.78



Supplementary Figure S4.5. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S4.2** above.

Supplementary Table S4.3. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S4.6** below.

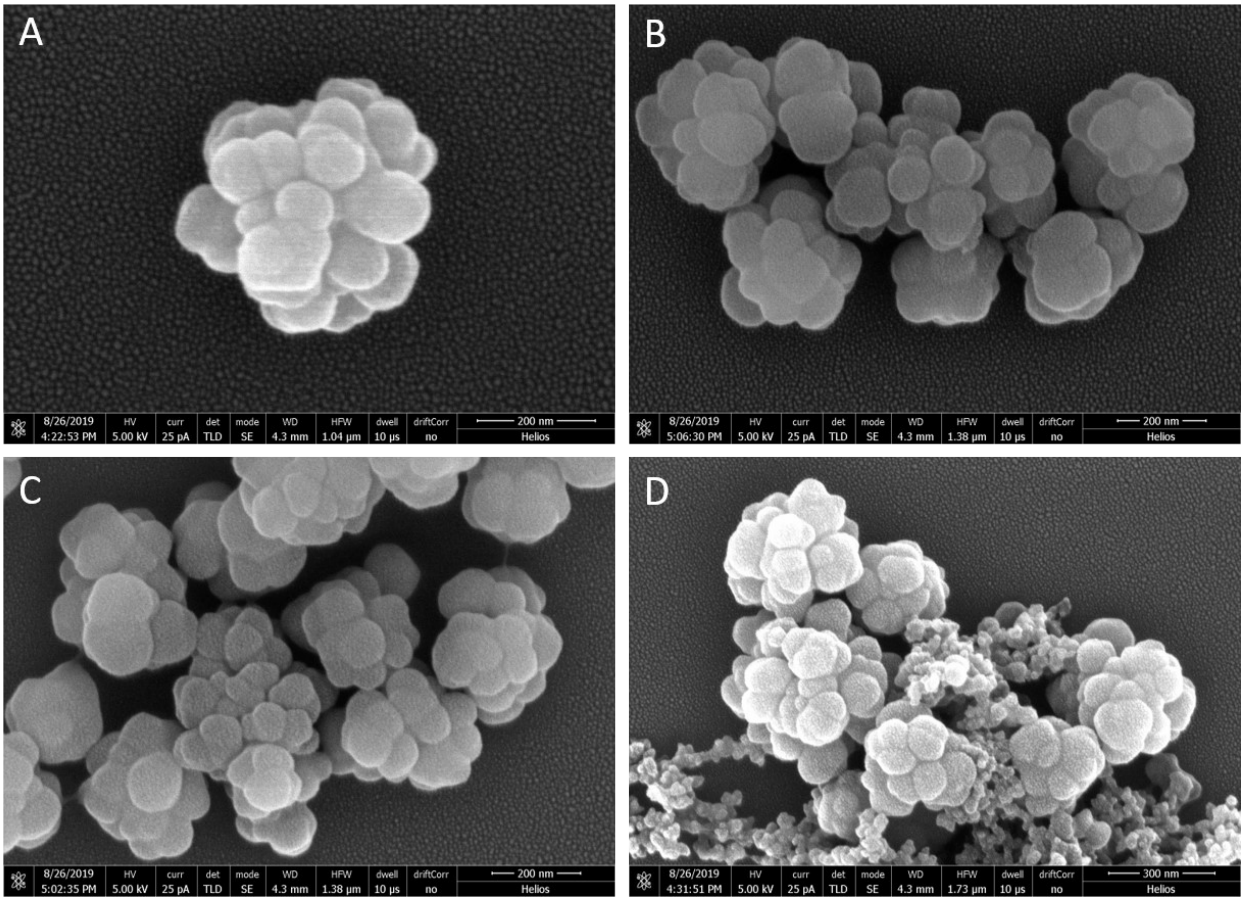
Aqueous Phase	2.5 mM PVP (55,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-17.2 ± 2.82



Supplementary Figure S4.6. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S4.3** above.

Supplementary Table S4.4. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S4.7** below.

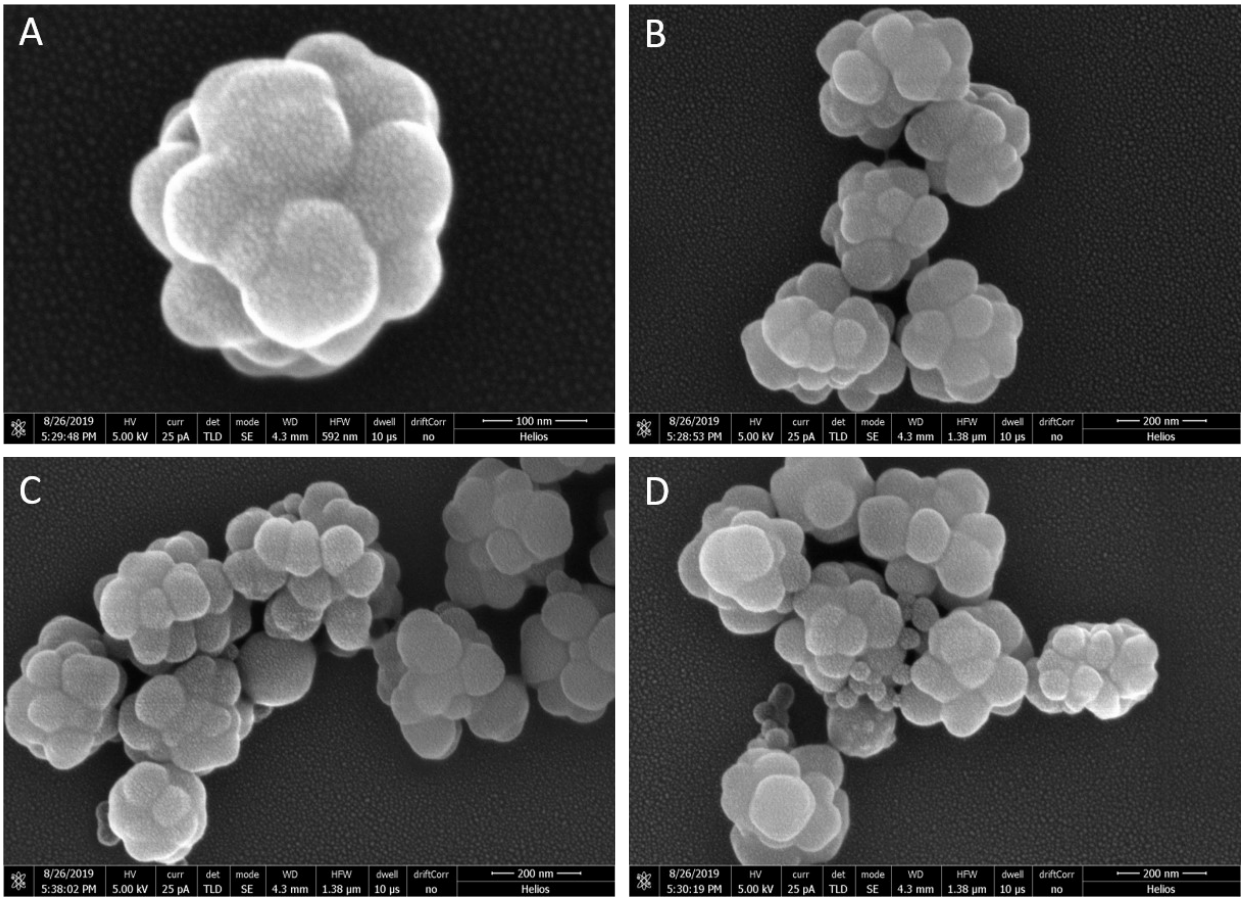
Aqueous Phase	1 mM PVP (55,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-15.8 ± 3.06



Supplementary Figure S4.7. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S4.4** above.

Supplementary Table S4.5. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S4.8** below.

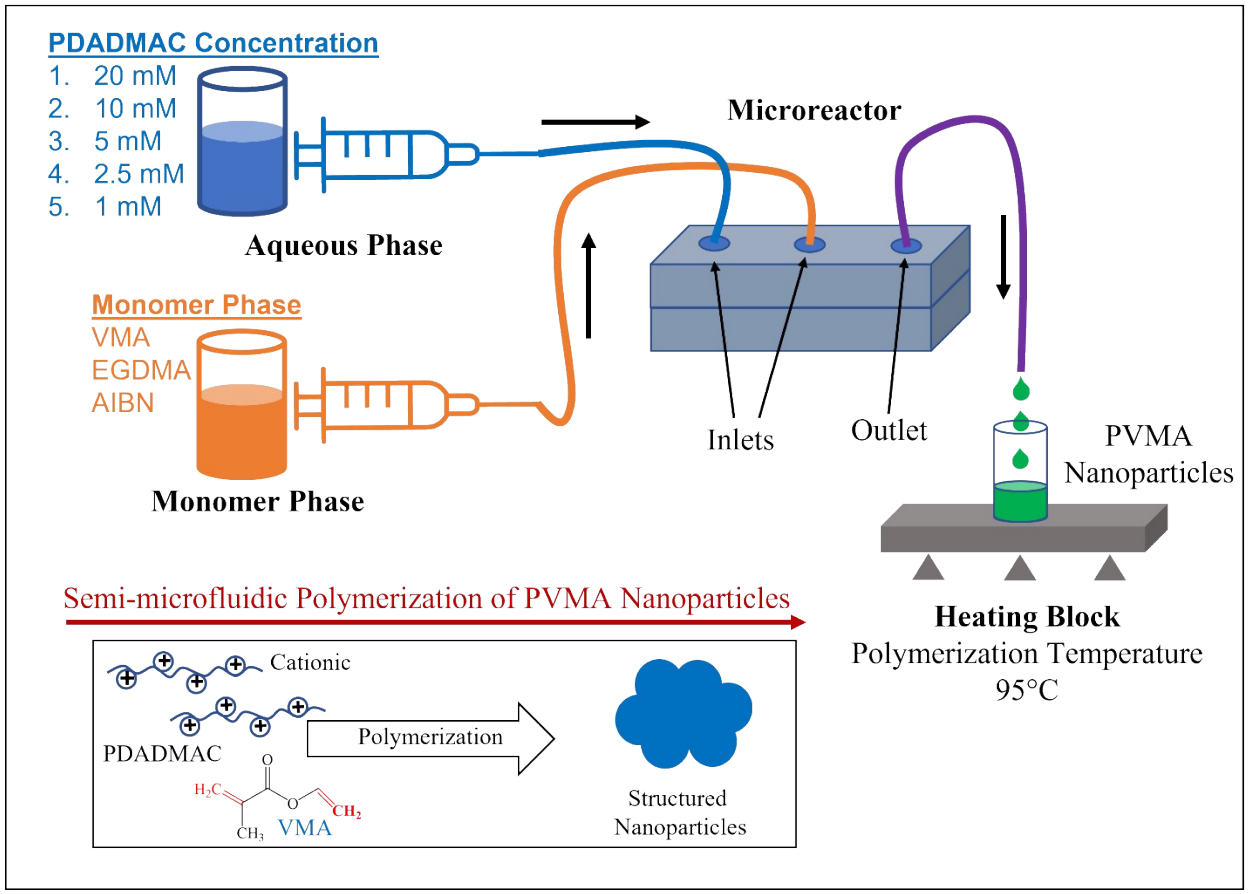
Aqueous Phase	0.5 mM PVP (55,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	-16.7 ± 3.25



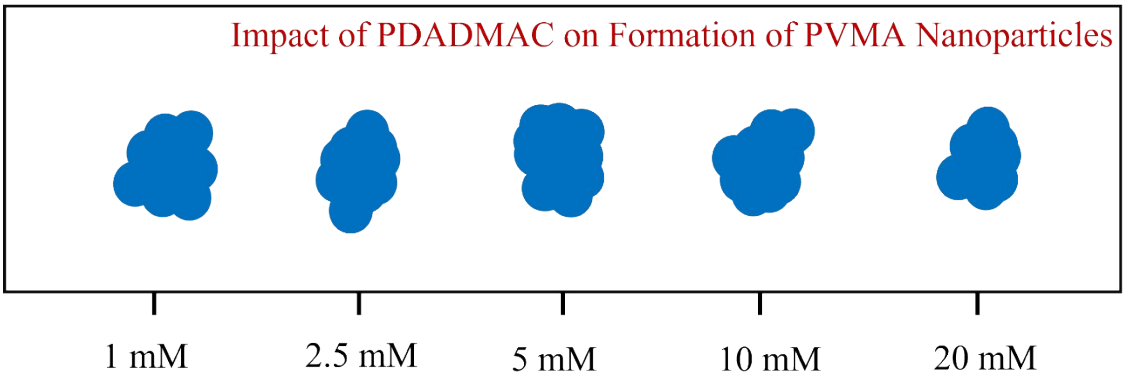
Supplementary Figure S4.8. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S4.5** above.

5. PVMA Nanoparticles: *Impact of PDADMAC*

Like PVP, the cationic polyelectrolyte PDADMAC also contributing to the impact where irregularly assembled anisotropic PVMA nanoparticles were obtained. The mechanism is also unclear in the case of using PDADMAC in the aqueous phase and further investigation is required. A basic reaction setup for the generation of anisotropic PVMA nanoparticles in presence of PDADMAC is shown in **Supplementary Figure S5.1**. Moreover, a general depiction of the obtained shapes of the PVMA nanoparticles at different PDADMAC concentrations is shown in **Supplementary Figure S5.2**.



Supplementary Figure S5.1. Reaction arrangement for the synthesis of PVMA nanoparticles by using PDADMAC in the aqueous phase. The effect of five different concentrations of PDADMAC (20 mM, 10 mM, 5 mM, 2.5 mM, and 1 mM—Repeating Unit Concentration) on the formation of PVMA nanoparticles have been investigated during different experiments.

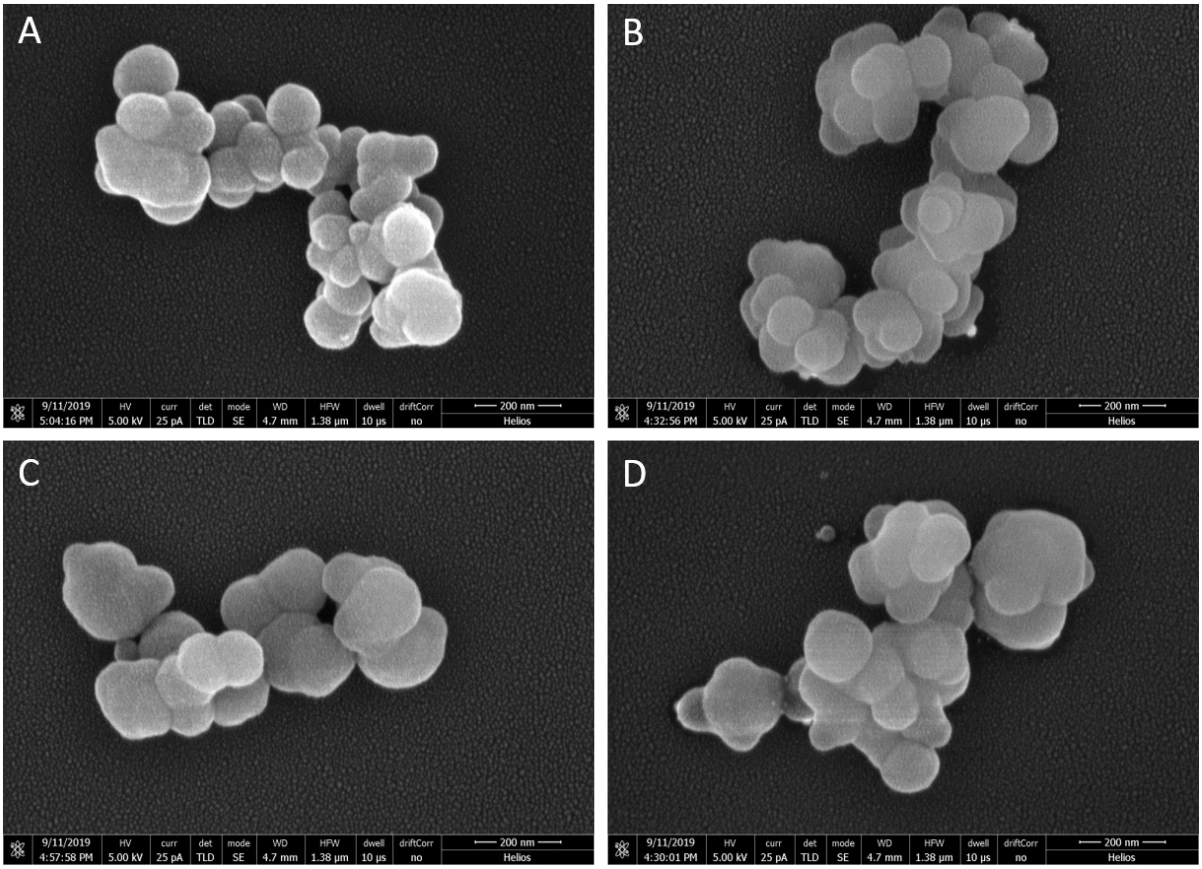


Supplementary Figure S5.2. Cartoons of the polymer nanoparticles were obtained at different PDADMAC concentrations in the aqueous phase during the polymerization process.

The detailed reaction conditions, zeta potential values, and SEM images of the obtained PVMA nanoparticles during application of PDADMAC in aqueous phase are shown in **Supplementary Table S5.1-S5.5** and **Supplementary Figure S5.3-S5.7** below**.**

Supplementary Table S5.1. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S5.3** below.

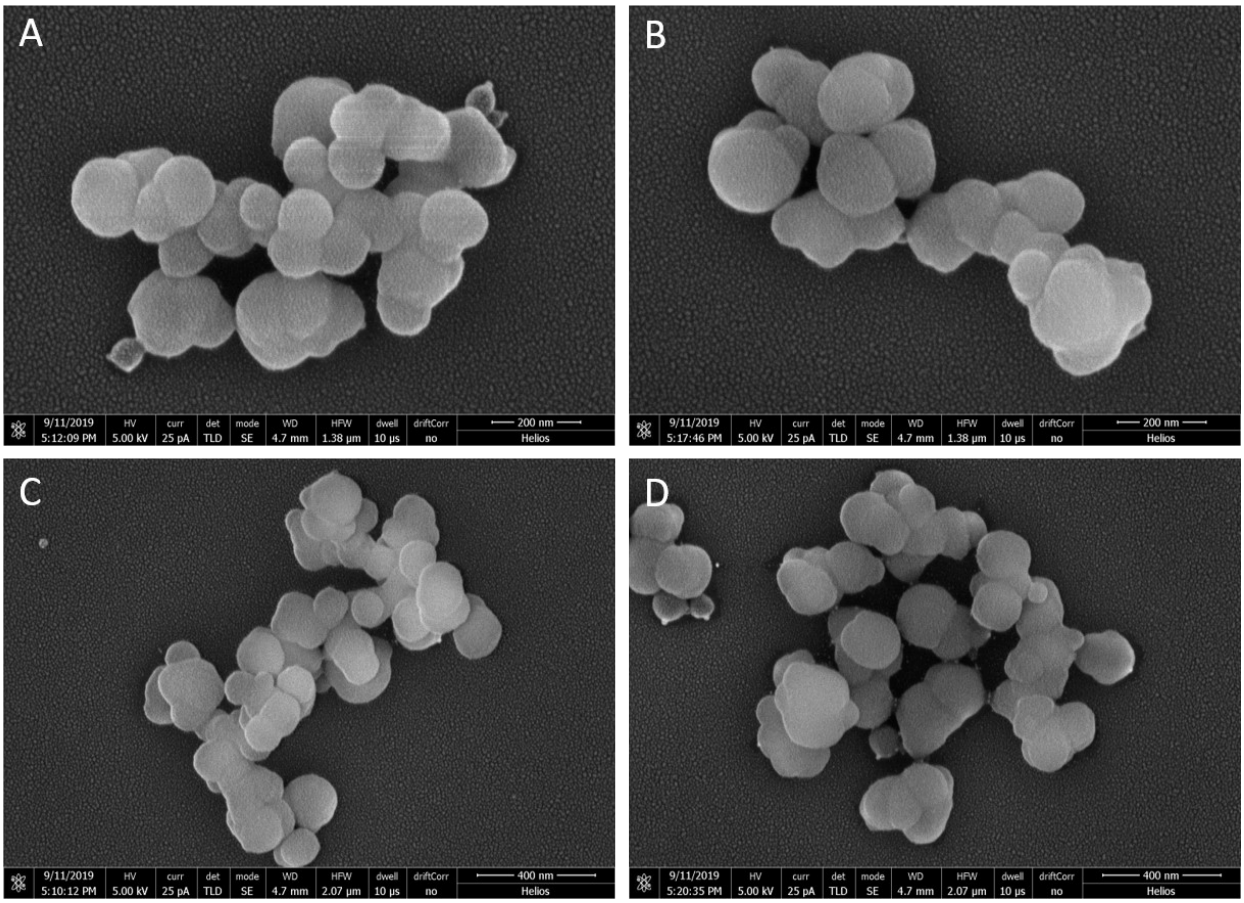
Aqueous Phase	20 mM PDADMAC (200,000-350,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	64.6 ± 4.02



Supplementary Figure S5.3. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S5.1** above.

Supplementary Table S5.2. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S5.4** below.

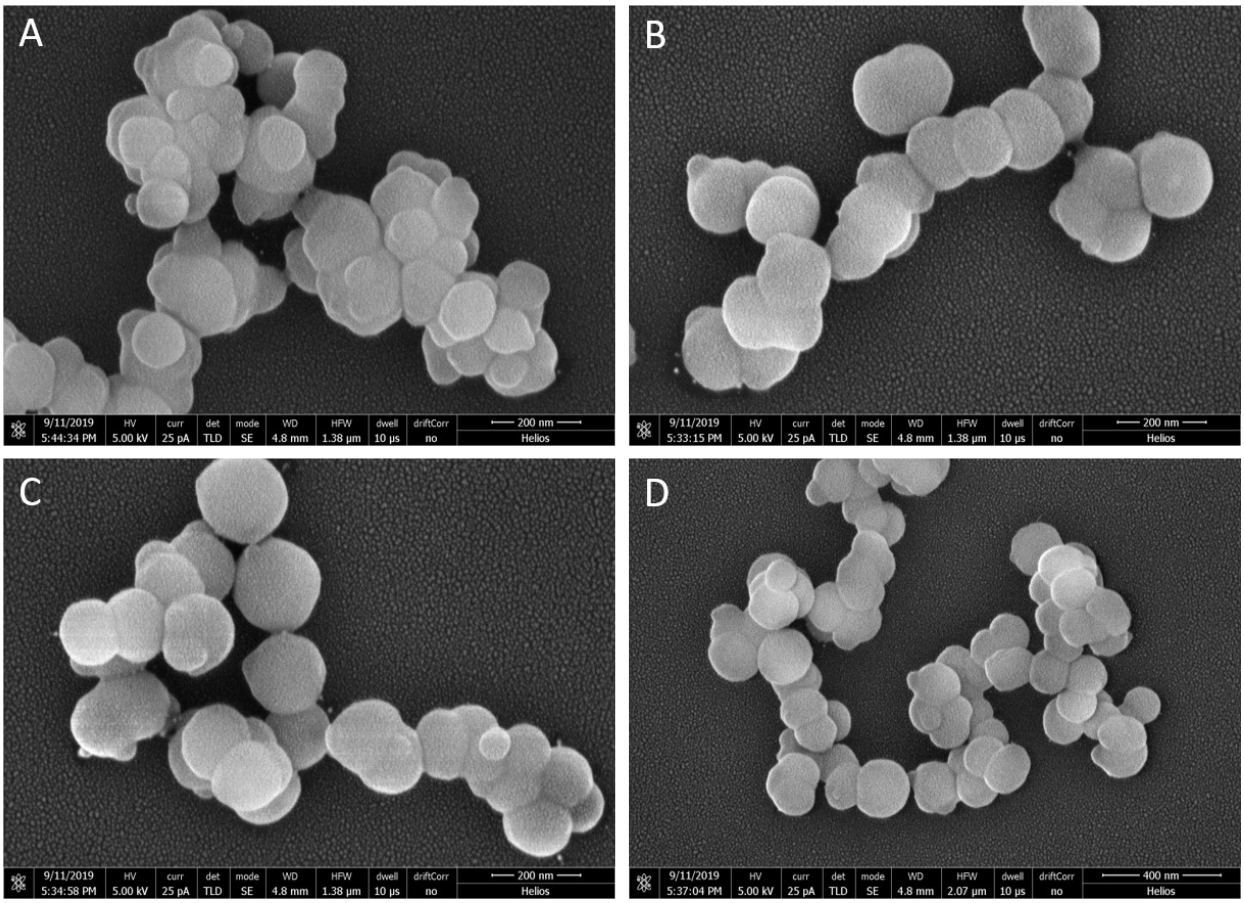
Aqueous Phase	10 mM PDADMAC (200,000-350,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	77.7 ± 3.95



Supplementary Figure S5.4. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S5.2** above.

Supplementary Table S5.3. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S5.5** below.

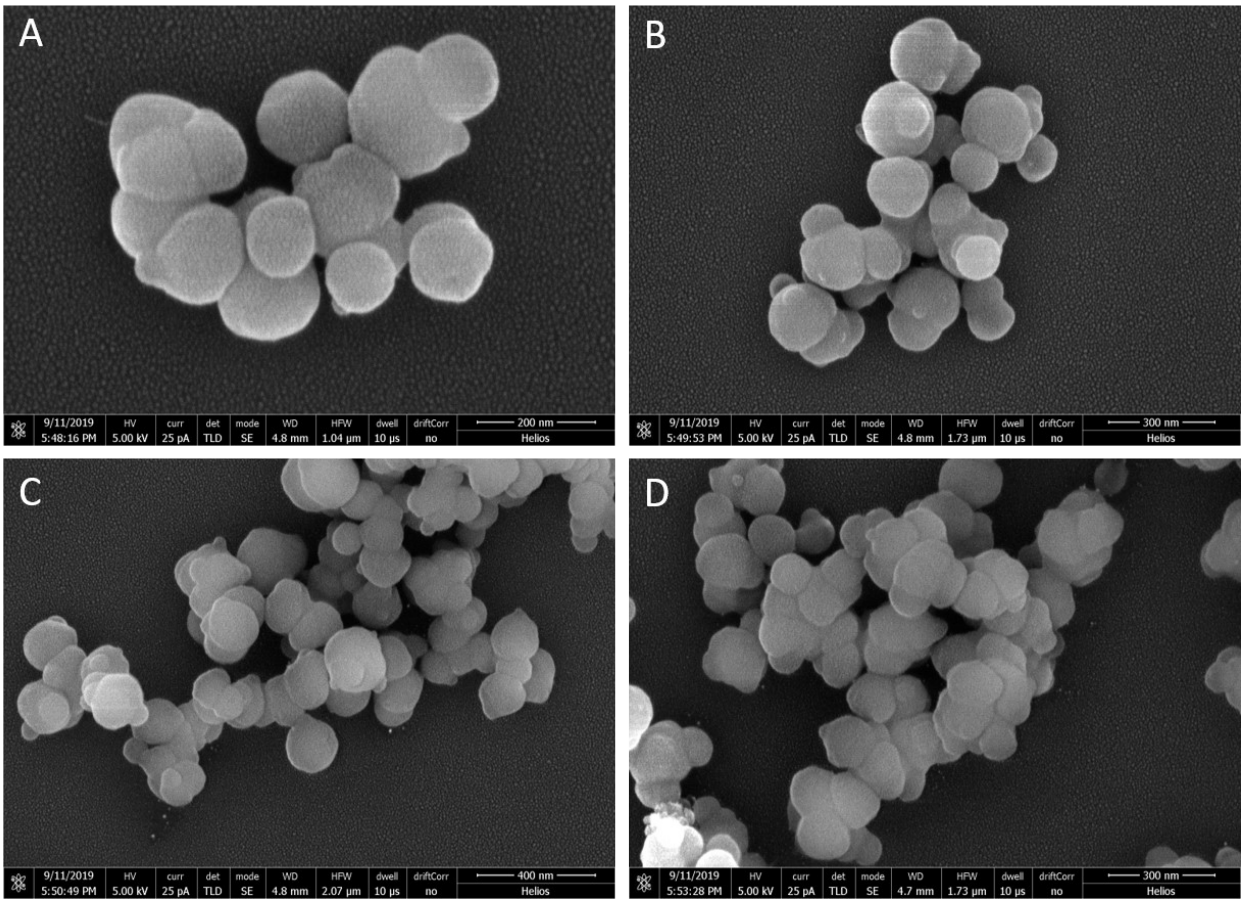
Aqueous Phase	5 mM PDADMAC (200,000-350,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	74.9 ± 3.76



Supplementary Figure S5.5. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S5.3** above.

Supplementary Table S5.4. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S5.6** below.

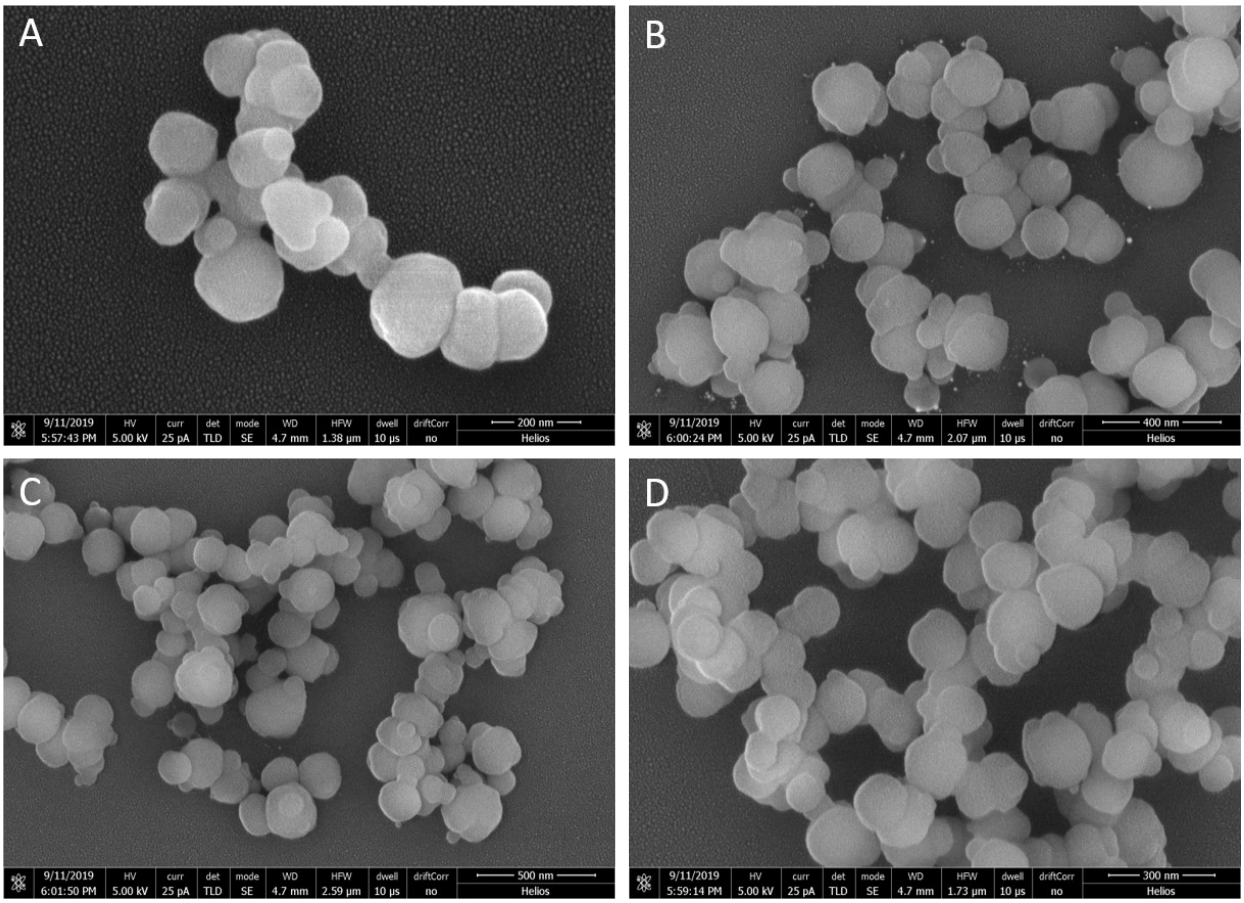
Aqueous Phase	2.5 mM PDADMAC (200,000-350,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	69.9 ± 3.94



Supplementary Figure S5.6. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S5.4** above.

Supplementary Table SZ5.5. Reaction condition, key responsible reactant, and zeta potential value of the obtained nanoparticles at given reaction condition in this Table. The obtained nanoparticles shown in this Table are shown in **Supplementary Figure S5.7** below.

Aqueous Phase	1 mM PDADMAC (200,000-350,000 molecular weight) (Repeating unit concentration)
Monomer Phase	990 µL Vinyl methacrylate (VMA) 10 µL Ethylene glycol dimethacrylate (EGDMA) 0.0040 g Azobisisobutyronitrile (AIBN)
Flow Rate ratio	1200/80 (Aqueous/Monomer Phase) (µL/min)
Temperature	95°C
Zeta Potential of Nanoparticles	63.6 ± 4.79



Supplementary Figure S5.7. SEM images of the PVMA nanoparticles. (A-D) Four SEM images of PVMA nanoparticles from four different locations for the reaction condition provided in **Supplementary Table S5.5** above.

In summary, shape-controlled nanoscale polymer particles becoming increasingly attractive for advanced biomedical applications such as phagocytosis, targeted drug delivery, and receptor-mediated endocytosis. Considering the soft nature of polymers, it is hard to mold the shape at nanoscale polymer particles during the single-step dynamic polymerization (solution-phase). The shape can be controlled *via a* multi-step process. However, desired functions throughout the polymer network are challenging to implement during the multi-step process. Therefore, a single-step approach is required where desired types of anisotropic polymer nanoparticles can be generated in a single step. Ideally, for real applications for healthcare purposes, biocompatible and biodegradable polymer nanoparticles are required. But biodegradable nanoparticles with desired shapes and other characteristics are hard to prepare. Also, there are many protocols are available where biocompatible nanoparticles can be generated in multi-step processes. But it is difficult to tailor the desired function *via* multi-step processes as stated above. Therefore, state-of-art techniques of the single-step anisotropic polymer nanoparticles are required (need to develop) for achieving better control over particles shapes. For the fundamental research, simple types of the monomer are best suited to study the shape parameter *via* single-step. Current

works are with relatively complex monomers and are best suited to obtain the single-step anisotropy in nanoparticles. Therefore, though the obtained anisotropic PVMA nanoparticles are not suitable for direct biomedical applications, their formation strategy through single-step opens the avenue to generate anisotropic polymer nanoparticles of more complex properties together with biocompatibility and biodegradability for direct biomedical applications in the future.