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Effect of processing parameters on the size distributions of comb polyelectrolyte stabilized complex coacervate microdroplets

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Effect of processing parameters on the size distributions of comb polyelectrolyte stabilized complex coacervate microdroplets

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Chemical Engineering

by

Zara Khan

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ABSTRACT OF THE THESIS

Effect of processing parameters on the size distributions of comb polyelectrolyte stabilized complex coacervate microdroplets

by

Zara Khan

Master of Science in Chemical Engineering

University of California, Los Angeles, 2024

Professor Samanvaya Srivastava, Chair

Complex coacervate microdroplets can be stabilized by comb polyelectrolytes and used as encapsulants for biological molecules such as proteins. In this thesis, we aim to create complex coacervate microdroplet dispersions with narrow size dispersity distributions by optimizing the concentrations and the molecular weights of homopolyelectrolytes and comb polyelectrolytes. The particle size distribution of suspended droplets is analyzed using static light scattering and microscopy. Further, we study the effect of mechanical processing (centrifugation and continuous stirring) on the particle size distribution of the complex coacervate microdroplets. Additionally, we investigate the reusability and recyclability of the complex coacervate microdroplet dispersions. Our process can potentially advance the use of complex coacervate microdroplets as synthetic reactor media for continuous stirred-type bioreactors.

The thesis of Zara Khan is approved.

Panagiotis D Christofides

Thaiesha Andrea Wright

Samanvaya Srivastava, Committee chair

University of California, Los Angeles

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List of Acronyms

PE - Polyelectrolyte

cPE - Comb Polyelectrolyte

PEC - Polyelectrolyte Complex

SLS - Static Light Scattering

PDADMAC - Poly(diallydimethylammonium chloride)

PAA - Poly(acrylic acid sodium salt)

PEG - Polyethylene glycol

M_w - Molecular Weight

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1. Introduction

Section 1.1. is reproduced with permission from Caitlyn Fick, Zara Khan, and Samanvaya Srivastava, "Interfacial Stabilization of Aqueous Two-Phase Systems: A Review." Materials Advances 4 (2023), 4665–78.

1.1. Polyelectrolyte Complexes

Aqueous two-phase systems (ATPS), consisting of two immiscible aqueous solutions, are routinely encountered in biology, chemistry, chemical engineering, and food science.[1–5] One of the first demonstrations of the utility of these systems was using dextran and methylcellulose for the separation of proteins by Albertsson in 1958.[6] Since then, ATPS have been utilized for the separation and purification of biomolecules,[1,5,6] in drug delivery,[7–9] as protocells and bioreactors,[10,11] detection of drug residues in food, drink, and water samples,[12,13] sequestration of precious metals,[14–16] and even for wastewater treatment.[1,3,17–19] These systems provide benign, biocompatible, and cheaper alternatives to oil–water systems, making them attractive materials systems for environmentally friendly manufacturing and processing techniques.[1,20–24]

The two phases in ATPS can consist of two immiscible aqueous polymer solutions, aqueous solutions of a polymer and a salt, two aqueous salt solutions, or a complex coacervate phase comprising oppositely charged polyelectrolytes in equilibrium with a polyelectrolyte-lean supernatant phase.[4,25–27] ATPS can be categorized as segregative or associative systems, depending on the thermodynamic drivers of phase separation.[11,25–27] In segregative systems, the

incompatibility between the aqueous solutions of the (macro)molecular solutes results in aqueous phases enriched in one solute (macro)molecule or the other.[1–3,25] A common, widely employed system is the mixture of polyethylene glycol (PEG) and dextran, which form a segregative ATPS with two phases comprising either PEG or dextran chains.[5,28] In contrast, associative ATPS form upon attractive interactions-driven complexation of (macro)molecules. Common examples of such systems include polyelectrolyte complex coacervates and membrane-less cellular organelles.[29–32] Segregative polymer–polymer ATPS have proven effective for separating many biological compounds and thus has become a familiar and frequently-used method in biotechnological settings.[6,21,33–37] The utility of ATPS for extracting biomacromolecules has been enhanced further by influencing the biomacromolecule to partition into either phase.[38,39] This can be done by affinity partitioning,[38–46] where the polymers themselves may be modified with affinity groups that are specific for biomacromolecules of interest[39–42,44,45] or by modulating the molecular weight of the polymers.[4,6,46–49] The physicochemical properties of the polymers, such as superficial electrochemical charge and hydrophobicity, can also affect the partitioning of biomacromolecules.[3,44,47,50] Polymer–polymer ATPS have been utilized not only for the separation and purification but also for the eventual extraction and encapsulation of proteins,[51] nucleic acids,[52] viruses,[53] antibodies,[39,54] and even whole cells.[43,45,55–60] These examples describe the diverse utility of polymer–polymer aqueous two-phase systems.

Polymer–salt systems are another common way to create segregative ATPS, wherein salts with high ionic strengths, such as phosphates, sulfates, and citrates, are most often used in combination with PEG and result in coexisting salt-rich and polymer-rich phases.[4,34,61] These systems are often used for cell encapsulation using microfluidic platforms, where droplet sizes and behaviors can be precisely manipulated.[57] Polymer–salt systems have also been used to separate proteins,[62–64]

DNA,[65] and virus-like particles[66,67] in systems such as PEG–sodium sulfate or phosphate, in addition to the encapsulation of cells[57] and purification of antibodies and biopharmaceutical products.[23,35,37,68] Salt–salt systems or ionic liquid–salt ATPS have also emerged in recent years as segregative ATPS.[4,61,69,70] These systems are formed upon the co-dissolution of a salt with strongly charged ions with another salt or ionic liquid comprising low-charge density ions.[4,61,70] Greater tunability of polarity enables these systems to overcome some of the issues, such as slow phase separation, found in polymer–polymer ATPS.[4,61] Therefore, extraction and purification abilities can be enhanced in salt–salt ATPS, encouraging their use for extraction and purification of biomolecules when in solution with secondary compounds or contaminants.[61,71,72]

Associative ATPS, in contrast, are driven by the complexation of (macro)molecules via attractive interactions and include systems such as polyelectrolyte complex coacervates and membraneless cellular organelles.[29–32,73,74] In the former, oppositely charged macroions (typically polyelectrolytes) undergo complexation, creating a macroion-rich complex coacervate phase separating from a macroion-lean supernatant phase (Fig. 1D).[29–32] In the latter, intrinsically disordered proteins (IDPs), or proteins with intrinsically disordered regions (IDRs), undergo phase separation in the cytoplasmic milieu, forming membraneless organelles such as nucleoli, stress granules, and P-bodies.[74,75] While attractive electrostatic interactions and the entropy gains from counterion release are the primary drivers for complex coacervation, the formation of membraneless organelles also benefit from hydrophobic collapse, hydrogen-bonding, π–π stacking, van der Waals interactions, and other short-range attractive intermolecular interactions to drive phase separation.[73,74,76–78]

Complex coacervates comprising oppositely charged polyelectrolytes are fascinating systems with immense potential for many different applications ranging from their use as biomaterials for

cartilage mimics, adhesives for wound healing, and drug delivery vehicles to their use as colloidal bioreactors in cell-free biocatalysis,[79,80] encapsulants in cosmetics,[81,82] and sorbents in wastewater treatment processes.[17,83,84] Concomitantly, a significant body of research investigating the thermodynamics of complexation as well as on the influence of diverse intrinsic properties (such as the polyelectrolyte chemistry,[85,86] length,[31,87] architecture,[32,86] charge density,[88–90] etc.) and extrinsic properties (ionic strength,[32,87] pH,[32,86,91] solvent quality,[85] temperature,[92] etc.) on the composition and properties of the coacervate and supernatant phases has emerged.[93] The crowded interiors of the coacervates and their ability to sequester charged (bio)macromolecules from their surroundings have promoted their utility as encapsulants and stabilizers in biological,[79,94] industrial,[95–97] environmental,[17,19,83] and cosmetic applications.[81,82]

Stabilization of the aqueous two-phase systems, including those such as polymer/polymer, water/ionic liquid,[98,99] and polyelectrolyte complex systems,[80] at the micrometer length scale or smaller, can be expected to enhance their utility even further by enabling the implementation of ATPS in a host of novel applications.[1,10,25,26,100] The canonical two-phase mixture of oil and water derives its significant utility from the stabilized mixtures in the form of emulsions and micellar solutions. These have inspired efforts for stabilizing water–water (w/w) interfaces, resulting in progressively increasing research attention. While the design of the interfacial stabilizer depends on the nature of the w/w interface and the composition of the two aqueous phases, a few broad themes can still be identified. As such, the energetically favorable coalescence (that reduces the interfacial area) must be superseded to stabilize droplets of one phase in another. This is typically accomplished by adding a third component that accumulates at the interface, reduces the interfacial area, and stabilizes the droplets. [101,102] The energy barrier that stabilizes the droplets is estimated

as the product of interfacial tension and the area taken up by the stabilizing components and is referred to as the trapping energy. In w/w interfaces, the interfacial tension is low, and correspondingly the trapping energy is significantly lower as compared to oil–water systems. Therefore, unique design approaches that go beyond employing surfactant-like molecules (with one anchoring point at the interface) for stabilizing the w/w interfaces must be developed. At the same time, the design criteria need to encode an affinity for both phases of the specific ATPS in the different parts of the stabilizer to promote its interfacial assembly.

The effectiveness of w/w interfacial stabilizers is typically ascertained by microimaging (to monitor the timespans over which droplets resist coalescence) and turbidimetry (to monitor the temporal evolution of the emulsion turbidity as a proxy for emulsion stability). The temporal evolution of the droplet size distribution can also be monitored to ascertain droplet stability. It provides insights into how the size distribution may affect the ability of the droplets to be stabilized. However, it is more often that researchers consider and modify the volumes of polymer or salt solutions or the size of stabilizing particles instead, which can ultimately affect the size of the droplets.[80,103,104] For example, it has been found that native proteins are generally too small for stabilization purposes (radius of ~2 nm).[101] It has also been reported that droplets stabilized by triblock polymers can experience changes in stability based on droplet size. In this case, smaller droplets with diameters of a few micrometers are more stable than those with sizes of the order of tens of micrometers.[105] It may be noted that the coalescence time varies significantly for different systems. The coalescence time can be affected by Brownian diffusion or the height of the energy barrier to form a hole in the stabilizing shell, which is influenced by the extent to which the droplet is covered.[106] Additionally, while the interfacial energy is minimized in these systems, it is not zero. Thus, the droplets will eventually coalesce to reach their lowest energy state.

In this thesis, we investigate the particle size distribution of complex coacervate droplets stabilized by comb polymers [80] and the effect of processing parameters such as rate of mixing, and fabrication techniques. In this following section, we discuss complex coacervate microdroplets and their application in encapsulating small molecules. Further, we discuss the role of comb polymer in stabilizing PEC droplets and maintaining an optimum size.

1.2. Complex Coacervate Microdroplets and Applications

Polyelectrolyte complex coacervate microdroplets are membrane-less compartments that can encapsulate small biomolecules and enhance their applications.[107] The associative aqueous phase complexation forms coacervate droplets that act as protocells encapsulating the protein globule in its native environment. The droplets provide an aqueous interface due to their membrane-less property i.e. a water-water interface that allows free movement of the ions[108] mimicking the native cellular milieu for encapsulated biomolecules. Unlike traditional liposomal or polymeric capsules with distinct hydrophobic membranes, coacervate droplets allow a free exchange of ions, small molecules, and substrates across the droplet interface. This feature is particularly beneficial for encapsulating and retaining the structural integrity and functionality of proteins and enzymes, which often require an aqueous environment to maintain their native conformation and catalytic activity.[109,110] Moreover, the coacervate droplet interface can be tailored by carefully selecting the polyelectrolyte components, their charge density, and solution conditions (e.g., pH, ionic strength). This tunability allows for the modulation of the droplet's permeability, surface properties, and interactions with the encapsulated biomolecules, enabling the optimization of the microenvironment for specific applications. Some notable applications include:

1. **Drug Delivery**: Complex coacervate microdroplets can be engineered to encapsulate drugs or therapeutic agents, providing controlled release and targeted delivery to specific tissues or cells within the body.

2. **Microencapsulation**: Complex coacervate microdroplets serve as carriers for encapsulating sensitive materials such as vitamins, flavors, fragrances, and enzymes, protecting them from degradation or environmental factors. Complex coacervate microdroplets can also be utilized in environmental applications for encapsulating and delivering remediation agents or catalysts to contaminated sites, facilitating pollutant degradation and environmental cleanup efforts.[111]

3. **Biomedical Imaging**: Fluorescently labeled Complex coacervate microdroplets can be used as contrast agents in biomedical imaging techniques like fluorescence microscopy and ultrasound imaging for visualizing biological structures and processes.[114]

4. **Consumer Products**: Complex coacervate microdroplets incorporated into cosmetic and personal care products can enhance stability, texture, and performance, such as in creams, lotions, and shampoos. [112, 113]

5. **Food and Beverage**: Microencapsulated flavors, colors, and nutrients in complex coacervate microdroplets can be used in food and beverage applications to improve taste, appearance, and nutritional value, as well as to provide controlled release.[114]

1.3. Motivation for this Work

Polymeric coacervates can be used in various ways, as described earlier. In this thesis, we discuss the formulation of comb polyelectrolyte-stabilized complex coacervate microdroplets and the effect of processing parameters on their size distribution. While most approaches for stabilization

of complex coacervate microdroplets introduce a hydrophobic layer around the droplets for stability, Gao et al. have demonstrated the use of comb polyelectrolytes to effectively stabilize complex coacervate microdroplets while preserving their water-water interface (Figure 1).[80] Comb polyelectrolytes possess a linear backbone with ionizable groups as well as tethered side chains, allowing them to effectively stabilize polymer droplets through several synergistic mechanisms. Their charged backbone enables them to adsorb onto the droplet surfaces, while the extended side chains protruding into the continuous phase provide steric stabilization and prevent droplet coalescence by forming a protective barrier around each droplet. Further, systems stabilized by comb polyelectrolytes do not interfere with the spontaneous sequestration of proteins inside the droplet. These characteristics make complex coacervate droplets suitable for developing fundamental biological systems using synthetic protocells. [115] Designing and preparing a synthetic protocell – an artificially made microcompartment that mimics a living cell has caught a lot of scientific and industrial attention in the past decade. Multiple studies about membrane-bound cell compartments currently exist.[116] However, they struggle with incorporating multiple small molecules for cascade reactions and inefficient release of the molecule. More recently, coacervate droplets fabricated by liquid-liquid phase separation have also emerged as a membrane-free compartmentalization technique.[117] This thesis delves into the robustness of the stabilized coacervate dispersions, which is critical for the large-scale deployment of coacervate-based formulations as protocellular reactor media.

It is preferred that the droplets stay in a stable particle size distribution for uniform encapsulation and do not undergo temporal size evolution over time.[118] In our approach, we develop complex coacervate protocells stabilized by comb polymer in a consistent size range that can successfully encapsulate proteins for long-term stability and withstand mechanical processing.

1.4. Organization of this Thesis

The thesis is organized as follows:

Chapter 2 discusses the materials and methods required for fabricating polyelectrolyte complex coacervate microdroplets. The methods of characterization involved measuring the particle size using static light scattering, microscopy of the droplets, and the effect of processing using centrifugation.

Chapter 3 discusses the various optimizations that were performed over the compositions and components that formed a PEC droplet. These optimizations resulted in droplets with a narrow size distribution that were stabilized over 48 hours. The optimizations were performed by varying the composition of both polyelectrolytes and comb polymer, and processing techniques.

Chapter 4 discusses the overall summary of this thesis and our vision for further work. It highlights potential applications for the optimized coacervate droplets.

2. Fabrication of PEC- Comb Polymer Stabilized Microdroplets

2.1. Introduction

In this thesis, we demonstrate the preparation of polyelectrolyte complex (PEC) coacervate microdroplets comprising oppositely charged polyelectrolytes (PEs) and stabilized by comb polyelectrolytes (cPEs) to compartmentalize small molecules such as enzymes and proteins. We also want to maintain the size of the droplets in a consistent range and optimize the stability of the microdroplets. Comb polymers have a unique chemical architecture with a linear main chain and multiple tethered side chains. [80, 119, 120, 121] In the case of complex coacervate microdroplets, the cPEs provide steric stabilization to prevent coalescence and aggregation.

For our experiments, various PE and cPE concentration combinations were fabricated to optimize the particle size and stability. Polyacrylic acid-*comb*-polyethylene glycol (Glenium 7500) was used as the comb polymer. Gao et al. [80] have previously shown that Glenium 7500 can be used as an effective stabilizer for coacervate droplets that can retain their spherical microstructure for up to 4 months. This Chapter discusses the materials and methods used to create the PEC droplets and characterize their properties.

2.2. Materials

Polyelectrolytes poly(acrylic acid sodium salt) (PAA, = 5100 g/mol, powder), poly(diallydimethylammonium chloride) (PDADMAC, = 8500 g/mol, 28wt% solution) were purchased from Millipore Sigma, Polysciences Inc. respectively. Comb-polyelectrolyte polyacrylic acid-comb-polyethylene glycol (PAA-*comb*-PEG, M_w = 39467 g/mol, 26 at pH = 6, PEG = 3000 g/mol), commercially called MasterGlenium 7500 was provided by BASF.

2.3. Preparing PEC Droplets

PEC droplets were prepared by mixing PDADMAC (MW – 8.5k, 10% wt solution), DI Water, and PAA (MW – 5k, 10% wt solution) in the desired ratio. Samples were mixed by pipetting and stirred for 12 hours before carrying our SLS measurements (Figure 1). Initially, PE droplets with varying PE concentrations were formulated without any comb polymer to analyze the particle size distribution and stability of droplets without any stabilizing agent.

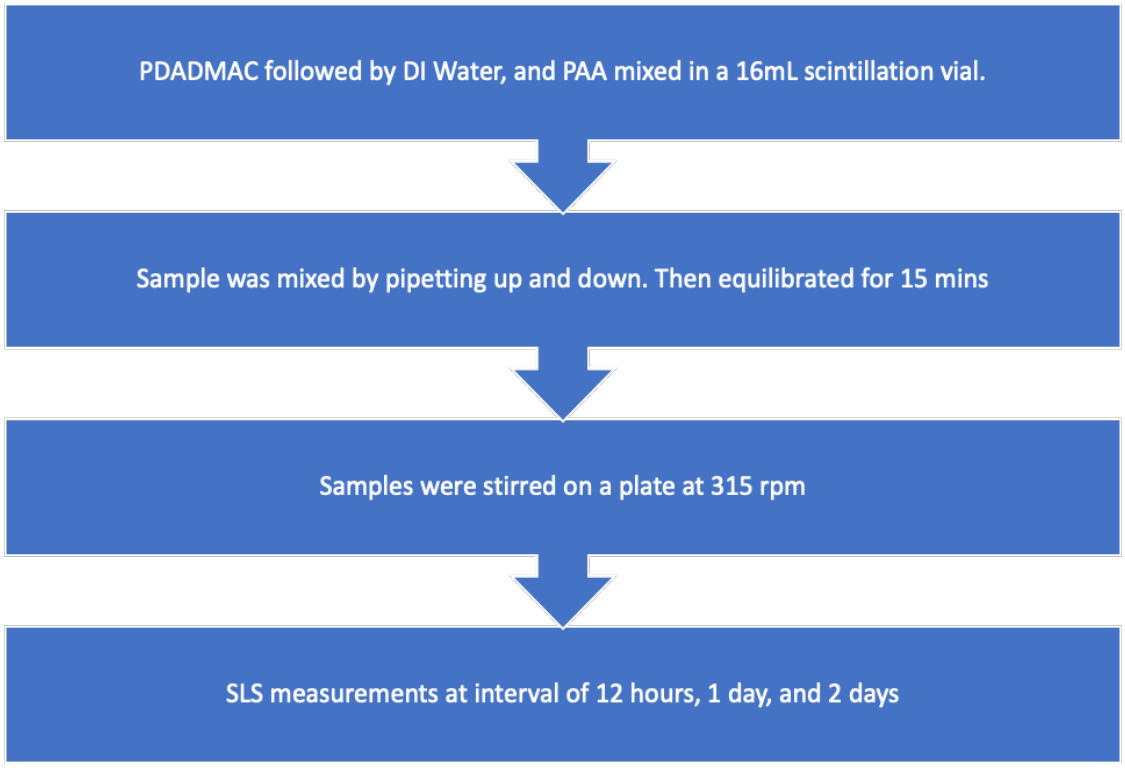

Figure 1: Protocol to formulate polyelectrolyte complex droplets using a continuous stirring method.

After varying the PE concentrations, samples were prepared with cPEs as a stabilizer. PDADMAC was mixed with Glenium, DI water, and PAA, in that order. Quantities for each component were calculated based on the total PE and cPE concentrations (Figure 2).

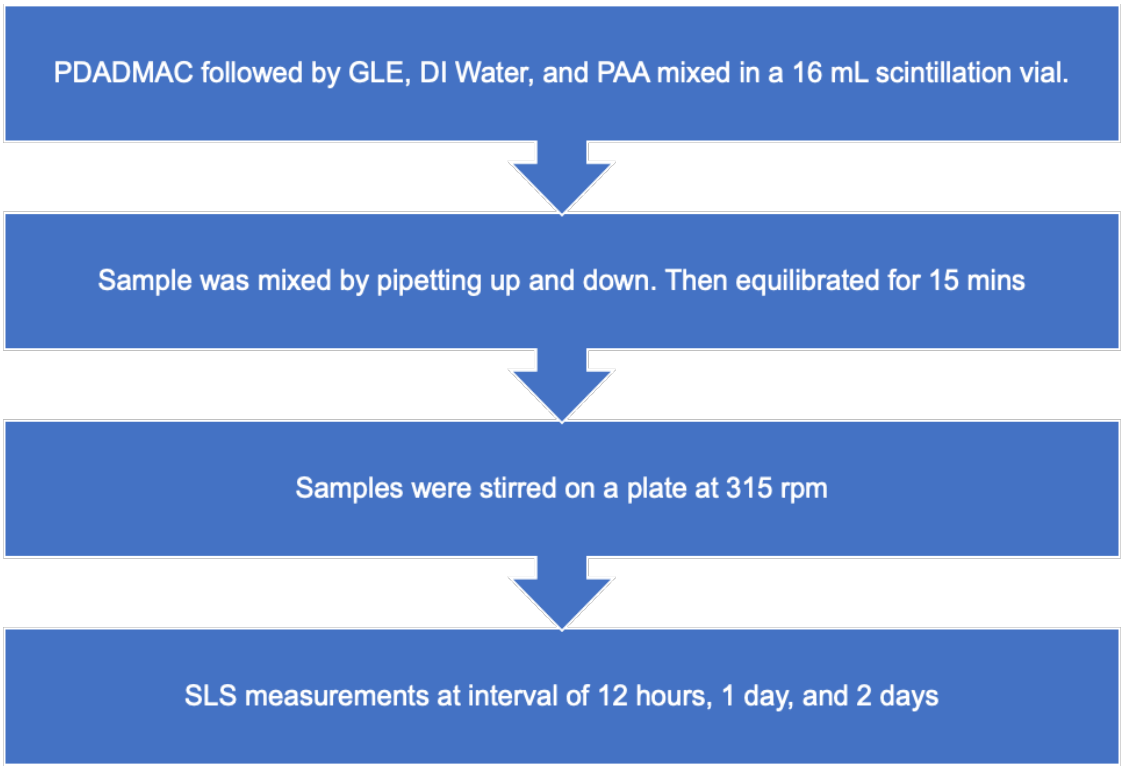

Figure 2: Protocol to formulate polyelectrolyte complex droplets with comb polymer using a continuous stirring method.

2.4. Static Light Scattering Experiment

Static Light Scattering (SLS), also known as Rayleigh scattering, is a technique used to analyze the properties of particles or molecules in a solution based on light scattering.[122] Unlike Dynamic Light Scattering (DLS), which measures the intensity fluctuations of scattered light over time, SLS examines the intensity of scattered light at a single point in time. The principle behind SLS lies in the interaction between incident light and the particles or molecules in the solution. When light encounters these particles, it scatters in different directions due to interactions with the particles' electric fields. The intensity of scattered light depends on various factors, including the particles' size, shape, and refractive index, and the wavelength and polarization of the incident light. [123, 124]

By measuring the intensity of scattered light at different angles relative to the incident light direction, SLS provides information about the particles' size and concentration in the solution. The intensity of scattered light is directly proportional to the square of the particles' radius and the square of the particles' concentration, making SLS particularly useful for determining particle size distribution and concentration in colloidal solutions. We used Horiba Partica LA-960V2 for our SLS measurements using custom software, LA960. Firstly, the machine was prepared for the sample input by ensuring the previous sample had been rinsed and drained. The method for the experiment was set by entering the appropriate refractive index for the sample and dispersing media. The transmission was maintained at 100% before sample input. An aliquot from the PEC samples (approx. 200 uL) was loaded one at a time into the system using a pipette. The sample was then ultrasonicated and de-bubbled. After these pre-processing steps, measurements were taken to create a distribution of q% (volume of droplets) vs diameter (um). Per the protocol, SLS measurements were done after 30 minutes, 12 hours, 1 day, and 2 days from fabrication.

2.5. Microscopy

The polyelectrolyte complex droplets were examined by microscopy. The slides were treated with AquaPel to obtain a hydrophobic surface and mounted on a stainless-steel mount. The Moticam microscope was set at a magnification of 40×. Three images were taken of each sample at multiple different positions to characterize the samples.

2.6. Redispersing PE Droplets after Centrifugation

While droplets were stirred, aliquots (1 mL) were taken and centrifuged. After centrifuging, the coacervate and supernatant were separated by pipetting. The coacervate was redispersed in the system after diluting in 1 ml of DI water. This process was repeated every 24 hours (Figure 3).

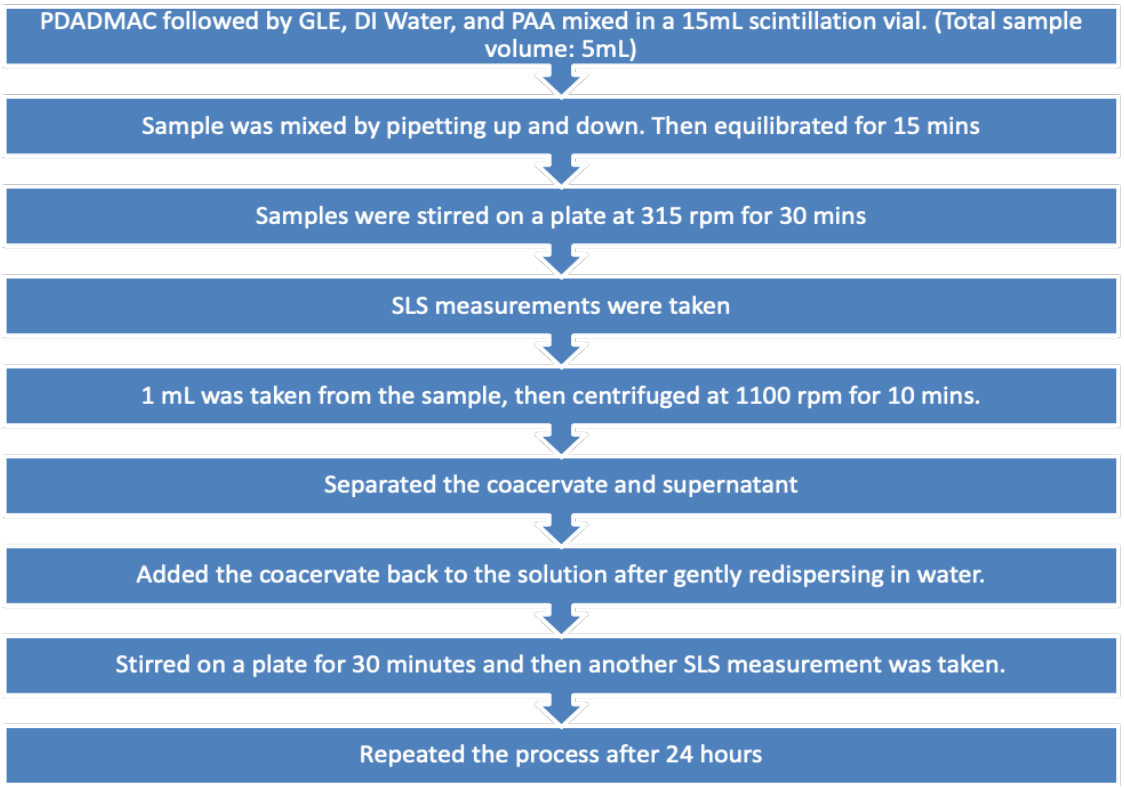

Figure 3: Protocol to fabricate polyelectrolyte complex droplets with comb polymer using a continuous stirring method and redisperse them in solution after centrifugation.

3. Optimization of PEC Droplets for Consistent Size and Stability

We aimed to fabricate PEC microdroplets in a consistent size range (<10 microns diameter) with numerous other properties, such as resistance to external stimuli and stabilization for 48 hours. Various polyelectrolyte concentrations, comb polyelectrolyte concentrations, and processing methods were tested to achieve the desired droplet characteristics.

3.1. Effect of Polyelectrolyte Concentrations on PEC Droplets

Various concentrations of polyelectrolytes were tested, ranging from 40 mM to 100 mM (Table 1). The total polyelectrolyte concentration was determined by combining both the polycation and polyanion (Table 1). The polyelectrolytes were charge-matched for each sample. SLS measurements done for samples (Figure 4A) right after mixing show that samples with PE concentrations 70 mM and 100 mM have a major volume of particles (15-20%) in the range of 1-10 microns diameter. 40 mM PE has most of the particles in the 40-70 microns diameter range. However, after stirring continuously for 24 hours (Figure 4B), and 48 hours (Figure 4C) we noted that a major volume of particles diameters are in the 20-100 microns range for all concentrations. For further experiments, we used 100 mM PE concentration as we saw the most consistent size distribution for this system in our initial tests.

PDADMAC Conc. (wt%)	PDADMAC Conc. (mM)	PAA Conc. (wt%)	PAA Conc. (mM)	Total PEC (wt%)	Total PEC (mM)
0.323	20	0.188	20	0.51	40
0.565	35	0.329	35	0.89	70
0.808	50	0.470	50	1.28	100

Table 1: Concentrations for preparing PE droplets of varying final concentrations

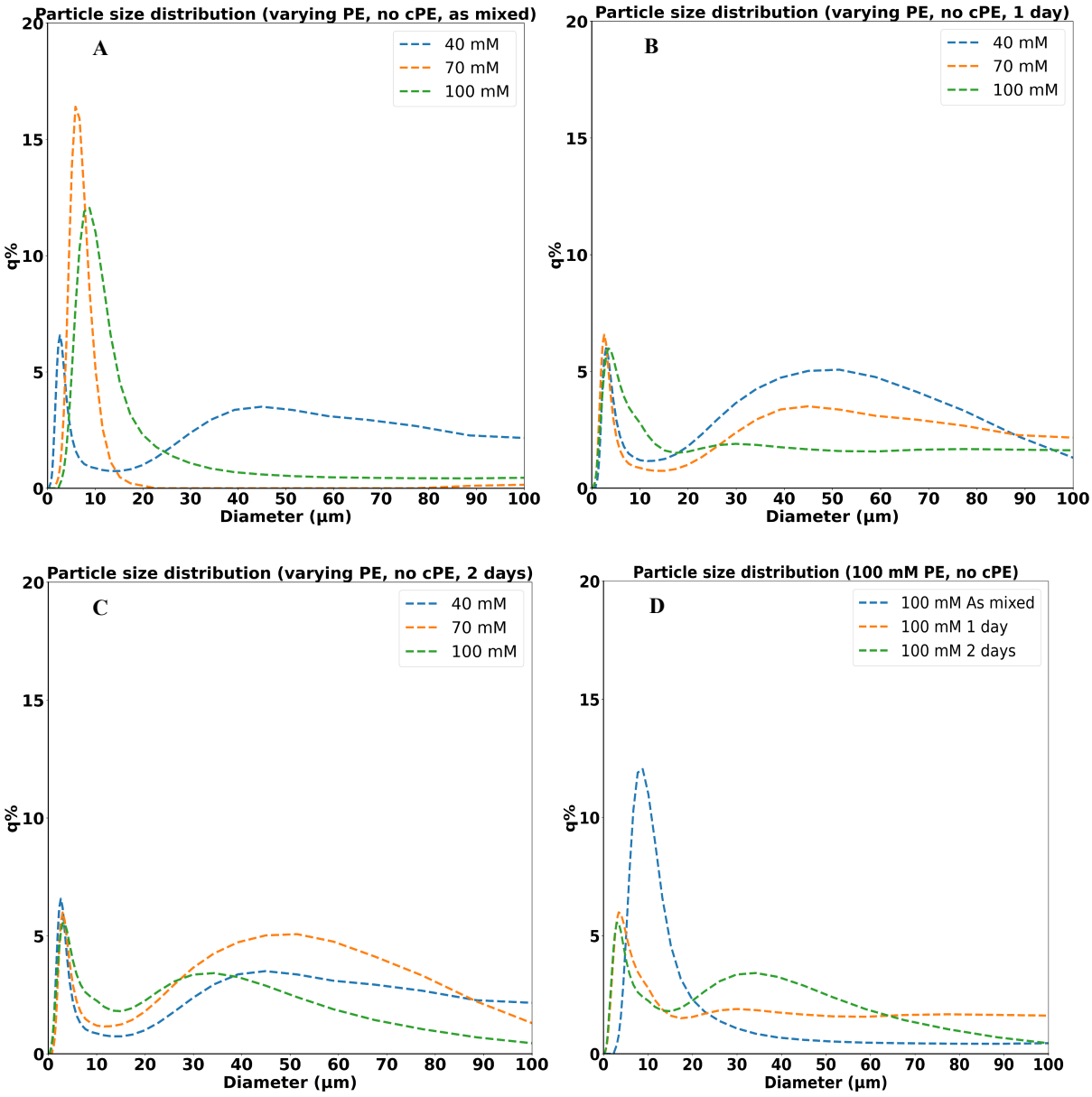

Figure 4: A-C) Polyelectrolyte (PE) complex droplets were formed without any comb polymer using various concentrations of PE. Through SLS measurements, we note that in the absence of any comb polymer stabilization the particle size varies significantly over a period of 2 days. A) Particle size distribution for 70 mM and 100 mM system is narrower compared to 40 mM system, B-C) After 1 and 2 days of continuous stirring, we noted that the size distribution for all samples is broad and particle diameter sizes vary from 2-100 microns D) Time analysis of particle size distribution for 100 mM PE over a period of 2 days *(q% represents the volume of droplets in the range)*

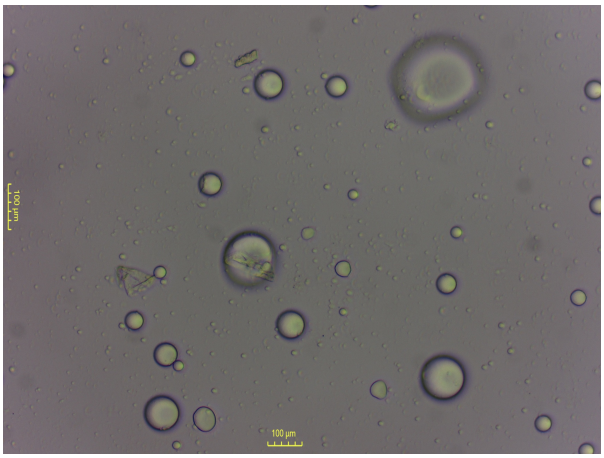

Figure 5: Microscopic image of polyelectrolyte complex solution with 100 mM total polymer content and no comb polymer. We noted droplet number density to be low and the droplets had varying particle diameter sizes, from as small as 10 microns to approx. 100 microns.

3.2. Effect of Comb Polymer Concentrations on PEC Droplets

Various concentrations of comb polymer (cPE) Glenium were tested, ranging from 2 mM to 50 mM with the different PE concentrations. Low concentrations of cPE (2-15 mM), and high concentrations (20-50 mM) were tested. For each trial, the ratio of PE:cPE was maintained at 10:1, 20:1, 5:1, 4:1, and 2:1 (Table 2). Preliminary visual inspection shows a difference in appearance between the PEC droplets made with and without comb polymer (Figure 7). Both samples contain 100 mM PE concentration, and the sample on the right has 20 mM Glenium 7500 added to it. The sample on the left (no cPE) is completely clear whereas, the sample on the right (with cPE) is turbid. Further, we noted from SLS measurements that samples with lower concentrations of cPE (Figure 7A) have a broader particle size distribution. A small volume of the particles (~10%) has sizes in the 2-10 microns diameter range. Samples with high cPE concentrations (20 mM-50 mM) have a narrow particle size distribution with over 20% volume of the particles in the 2-10 microns diameter range (Figure 7B). From microscopic imaging (Figure 7C-D), we noted that a higher density of droplets is present in the sample with higher Glenium concentration (20 mM) as

compared to the sample with a lower Glenium concentration (10 mM). Based on these results, we used a PE:cPE ratio of 5:1 or 4:1 for our further tests.

Total PE (mM)	Glenium (mM)	PE: cPE
100	5	20:1
100	10	10:1
100	20	5:1
100	25	4:1
100	50	2:1
70	7	10:1
70	14	5:1
40	4	10:1
40	8	5:1

Table 2: Polyelectrolyte- comb polyelectrolyte combinations tested with Glenium.

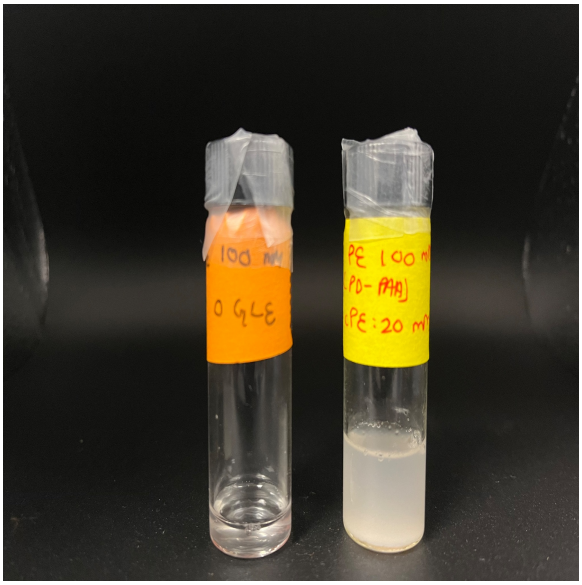

Figure 6: The sample with no comb polymer (left) is clear whereas complex with Glenium 7500 (20 mM) is turbid (right). Both samples contain 100 mM PE.

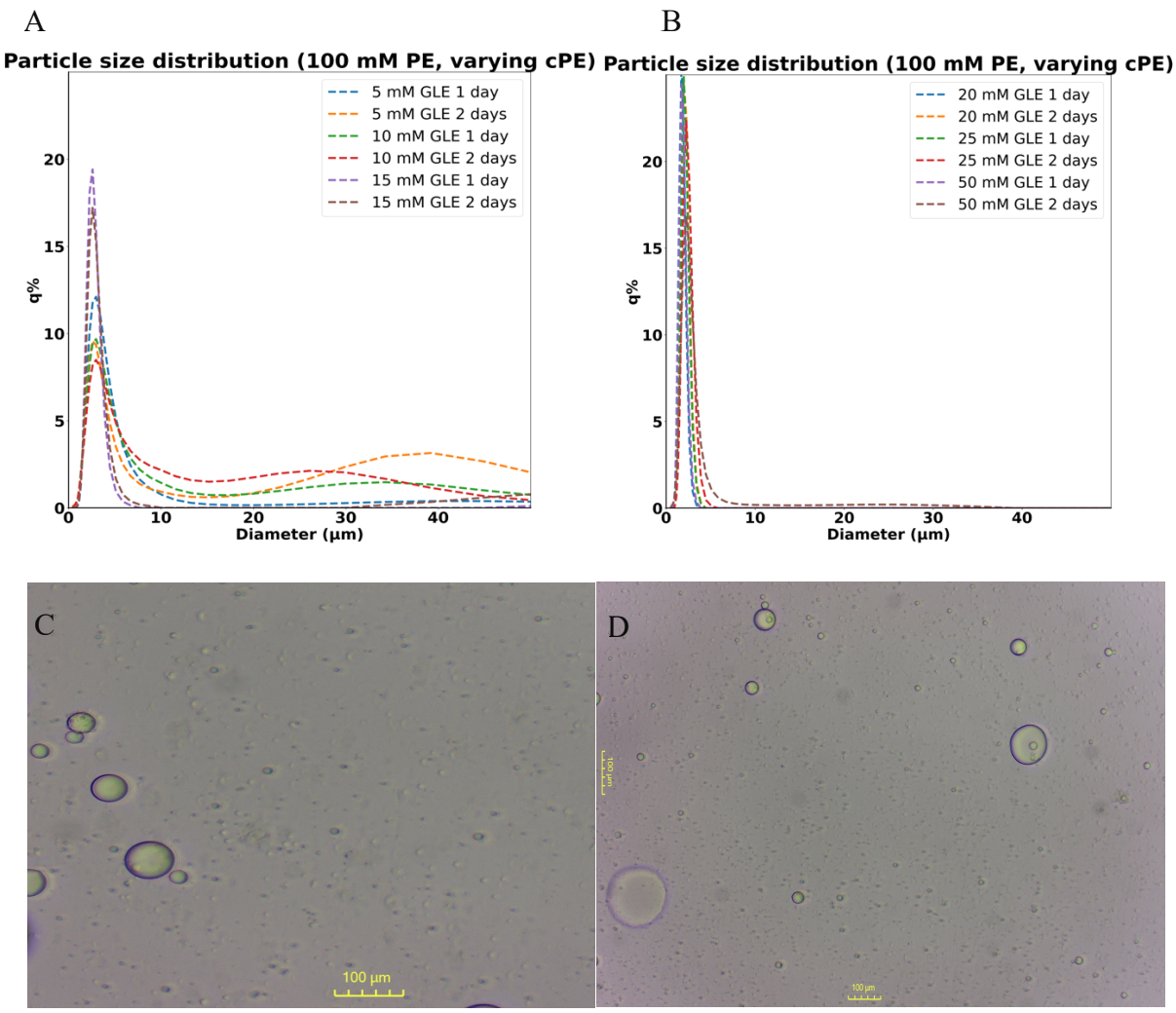

Figure 7: A-B) Polyelectrolyte (PE) complex droplets were formed with varying comb polymer concentrations using 100 mM of total PE; A) lower concentrations of cPE (5-15 mM), B) higher concentrations of cPE (20-50 mM). By SLS measurements, we noted that in low comb polymer concentrations the particle sizes are not evenly distributed, on the other hand, higher comb polymer concentrations have a monodispersed particle size distribution *(q% represents the volume of droplets in the range)* C-D) Microscopic image of polyelectrolyte complex solution with 100 mM total polymer content, C) 10 mM Glenium-7500, D) 20 mM Glenium-7500. We noted droplet number density to be high and droplets were mostly monodispersed.

3.3. Effect of Processing Methods

3.3.1. No Stirring or Centrifugation

Droplets were fabricated in a scintillation vial and then mixed by pipetting vigorously. Each sample had 100 mM PE concentration and varying cPE concentrations (Figure 8). The samples

were allowed to mix for 30 minutes. SLS measurements were done after 12 hours, 1 day, and 2 days of preparation. In initial SLS measurements, the droplets size distribution is narrow and nearing the optimum 2-10 microns diameter range. However, over time the size distribution becomes broad with much of the volume of droplets in the higher (20-40 microns diameter) size range (Figure 8).

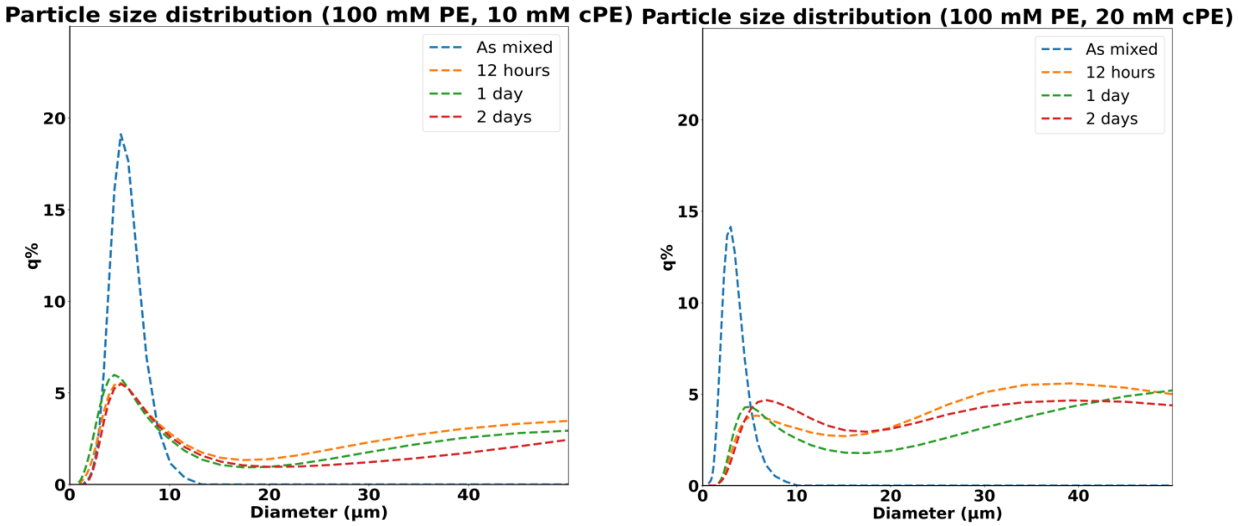

Figure 8: Droplets fabricated with no stirring, particle size distribution over time changes significantly for both A) 10 mM cPE (Glenium) and B) 20 mM cPE (Glenium). The size distribution is broad and goes well beyond the optimum < 10-microns range irrespective of the amount of cPE added. *(q% represents the volume of droplets in the range)*

3.3.2. Centrifugation

The droplets were prepared in Eppendorf tubes and then centrifuged for 10 minutes at various speeds to understand the change in particle size distribution. Each sample was prepared with a PE concentration of 100 mM and cPE concentration of 20 mM. SLS measurements show that the droplets had a narrow particle size distribution before centrifugation with particle size in the 2-10 microns diameter range (Figure 10A-C). After centrifugation, we noticed a significant change in the size distribution for all the samples, regardless of the time duration and speed of mixing. Most

of the droplets increased in size suggesting aggregation of the droplets (Figure 10A-C). A further study to corroborate this phenomenon can be planned.

Time (minutes)	Centrifuge Speeds (rpm)
2	800, 1000, 1200
5	800, 1000, 1200
10	800, 1000, 1200

Table 3: Various centrifuge speeds and time for the droplets

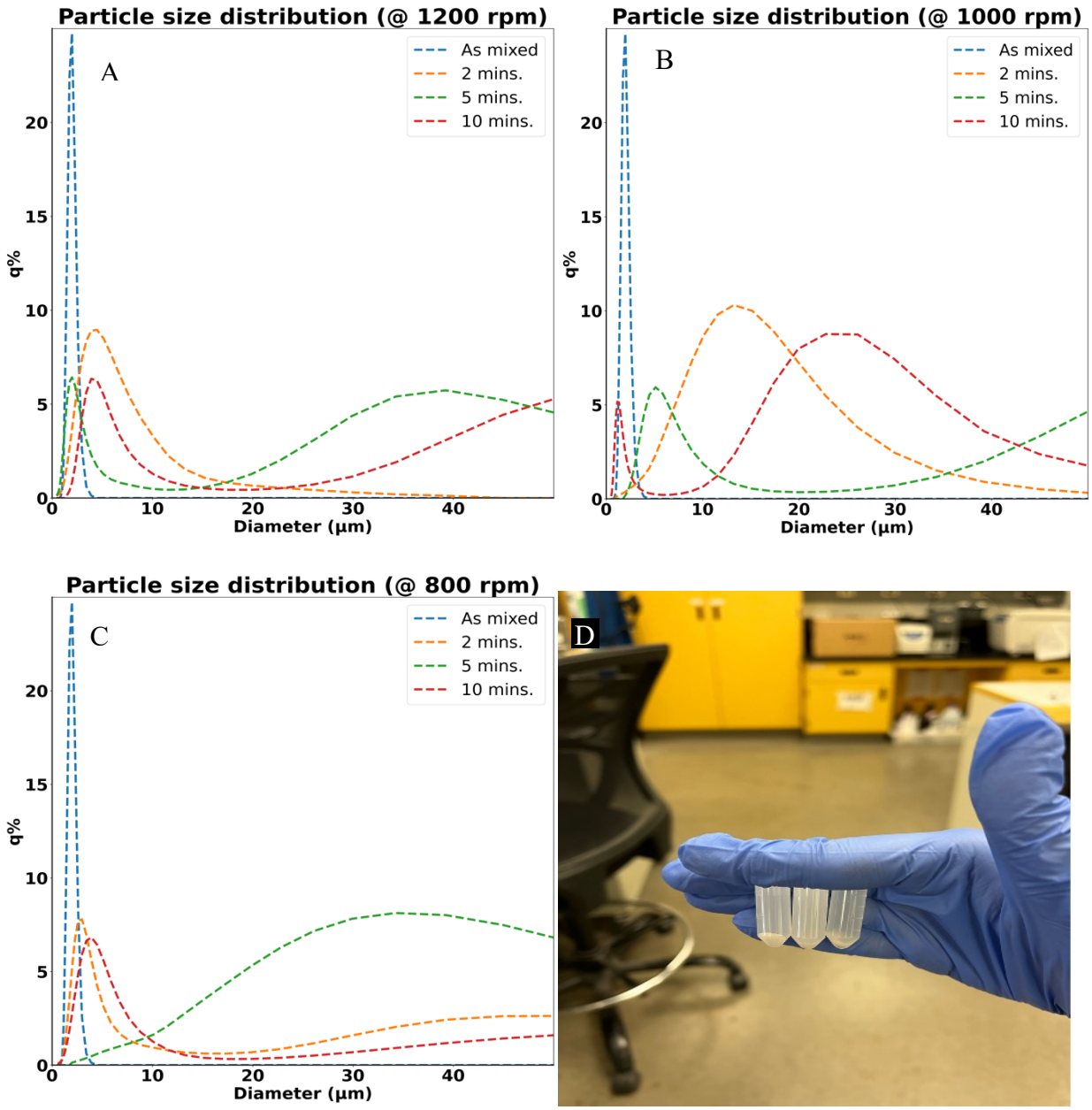

Figure 9: Droplets processed under different centrifuge conditions with PE 100 mM and cPE (Glenium 7500) 20 mM. A) 1200 rpm, B) 1000 rpm, C) 800 rpm; On initial SLS measurement (as mixed), the size distribution is narrow suggesting the formation of monodispersed droplets. In all cases, we note droplets size distribution is broad and not monodispersed after centrifugation. *(q% represents the volume of droplets in the range)* D) Droplets after centrifugation; the two distinct phases coacervate and supernatant are clearly visible

3.3.3. Stirring on Plate

Droplets were fabricated in a scintillation vial and then mixed by a magnetic stirrer on a magnetic hot plate at 315 rpm. The droplets were allowed to mix continuously on the plate. SLS measurements were done after 12 hours, 1 day, and 2 days of preparation.

This method was most successful in fabricating droplets in a monodispersed particle size distribution. All experiments done in section in section 3.1, 3.2, and 3.4 used this method for preparation.

3.4. Redispersing Coacervate Droplets after Centrifuging

We tried to understand the effect of processing parameters on the PEC droplets. We fabricated the droplets as outlined earlier using PDADMAC, PAA, Glenium and then centrifuged an aliquot (1 mL) at 1300 rpm for 10 mins. The supernatant was extracted from the coacervate after centrifuging. The leftover coacervate was redispersed in DI water by gentle shaking and then added back to the sample vial. SLS measurements were done before and after centrifuging. The cycle was repeated 2 times for each concentration of cPE. PE concentration was maintained at 100 mM for all the samples. We noted that the droplets with high concentrations of cPE (25- 50 mM) have a narrower size distribution compared to lower concentrations of cPE (10-20 mM) (Figure 11A-D). A major volume of droplets (~20%) possess a particle size in the 2–5-microns diameter range (Figure 11C-D). At lower concentrations, redispersed droplets are not as size stabilized (Figure 11A-B).

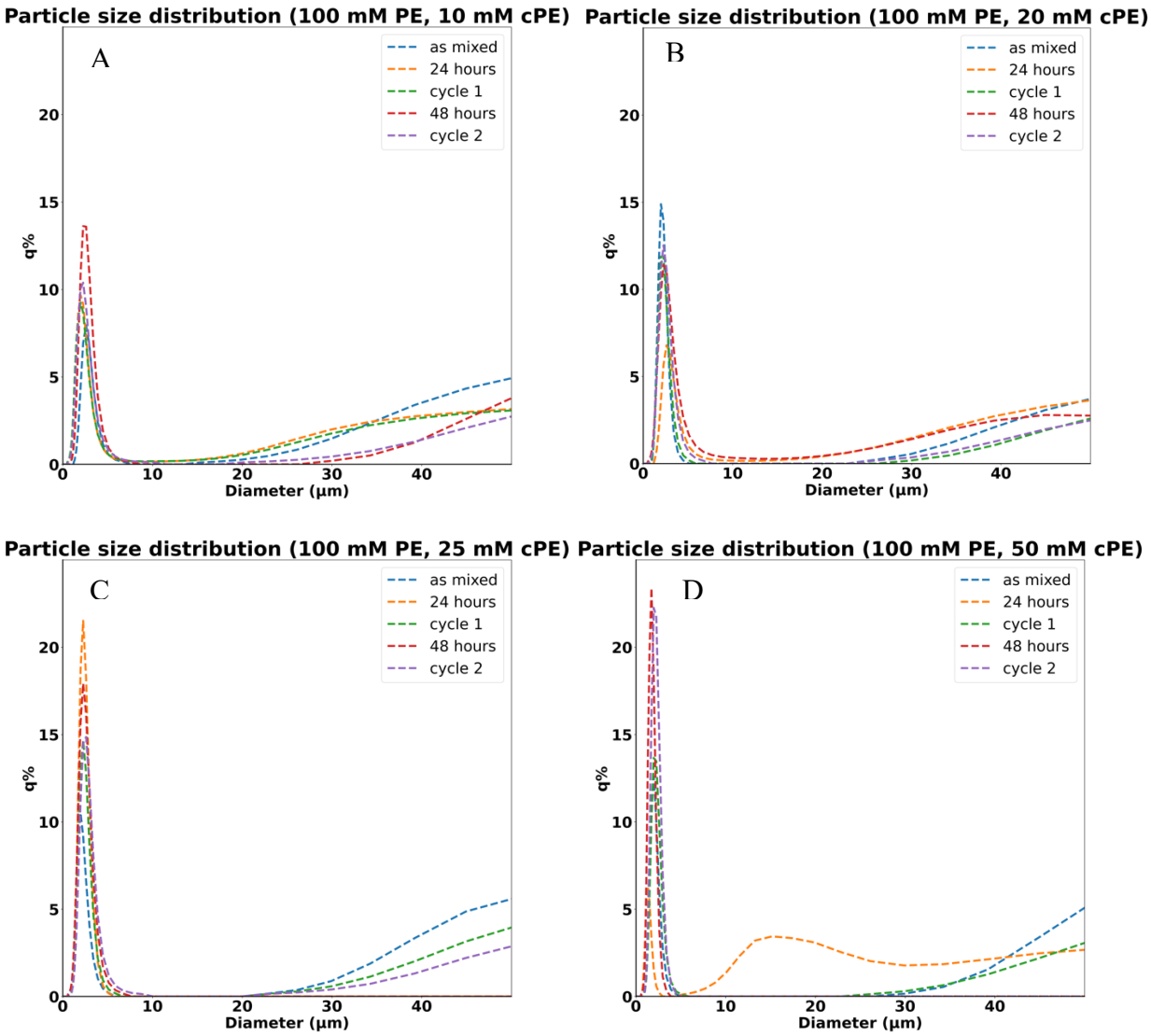

Figure 10: A-D) Redispersed droplets after 2 cycles, PE concentration was maintained at 100 mM with varying cPE (Glenium 7500) concentrations. We note that centrifugation doesn't change the particle size distribution of the droplets with higher cPE content when they are redispersed in the continuously stirring vial and are allowed to mix for over 12 hours *(q% represents the volume of droplets in the range)*

4. Conclusion

Stability of polyelectrolyte complex droplets can be enhanced by the addition of comb polyelectrolytes, and it can be optimized by varying the concentrations of both polyelectrolytes and comb polyelectrolytes. These microdroplets can attain a narrow particle size distribution by efficient mixing by techniques such as continuous stirring as illustrated in this study. The particle size range varies from 2-10 diameter which is an optimum size for encapsulation of small biomolecules such as enzymes and proteins.

To achieve a narrow particle size distribution in the 2-10 microns diameter range, the optimum concentration mix of PE and cPE is 100 mM and 25 mM, respectively. Throughout this study we used PDADMAC as polycation and PAA as polyanion with Glenium as the comb polymer. In future, other commercially available comb polymers can also be used as stabilizers.

We studied multiple processing methods to fabricate PE microdroplets as well as understand the change in their characteristics under different processing conditions. On centrifugation, the droplet size distribution changes significantly and leads to aggregation of droplets (particle sizes > 40 microns diameter). On the other hand, if the centrifuged droplets are separated into their coacervate and supernatant phase, and the coacervate is redispersed in the stirring vial, the samples regain their earlier size distribution (the desired narrow distribution) after thorough mixing for a period of 12-24 hours. This suggests that the droplets can be re-used in a continuous process promoting recyclability and sustainability.

We envision that these droplets can be used in multiple applications such as encapsulation of enzymes in the preparation of biofuels or in the production of perfumes which require small fragrant molecules to be encapsulated. Synthetic protocells have an advantage over other protocells

as they can withstand extreme temperatures and external stimuli, and do not need to tailor their environment unlike living cells.

As discussed in this study, polyelectrolyte complex microdroplets can compartmentalize small molecules and remain stable over extended periods of time. Further work on incorporating such a protocol for a larger setup can be investigated. Secondly, their use in a reactor can be studied with a focus on recyclability of feed streams and end products. These droplets can be tuned to have properties such as resistance to high temperature, pH fluctuations, pressure fluctuations, etc. Further, there is a need to develop a mechanism for effective encapsulation and release of the small molecules in the process.

5. Appendix

Appendix A:

SLS measurements of poleyelectrolyte complex droplets stabilized by low concentrations of Glenium 7500, measurements taken as mixed

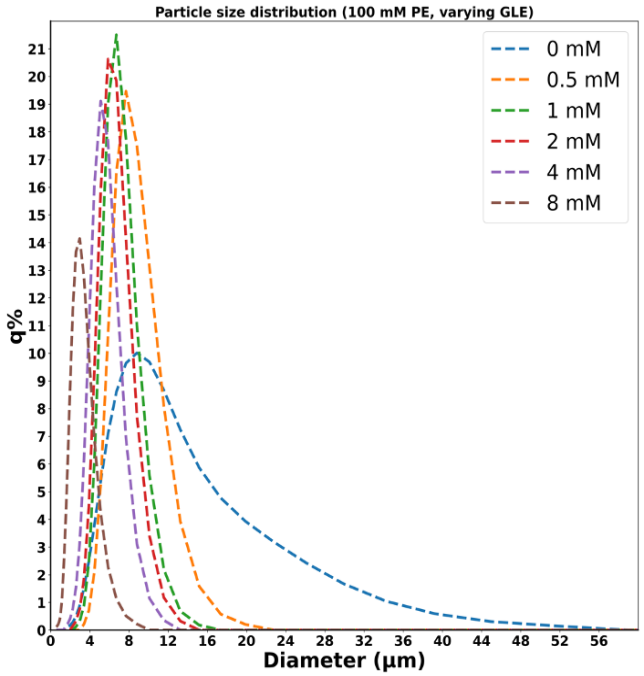

Figure A1: SLS measurements of poleyelectrolyte complex droplets stabilized by low concentrations of Glenium 7500, measurements taken as mixed

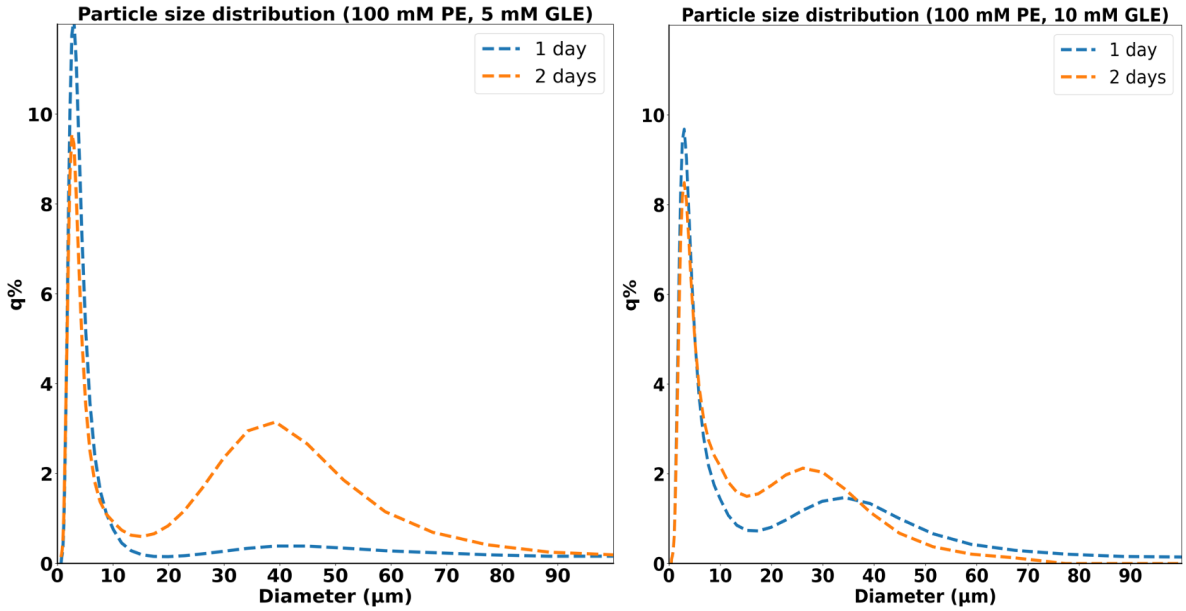

Figure A2: SLS measurements of poleyelectrolyte complex droplets stabilized by low concentrations of Glenium 7500, measurements taken over time

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