FORMATION OF COLLOIDAL DISPERSIONS OF POLYMER MATERIALS IN AQUEOUS MEDIA BY SOLVENT SHIFTING

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Thesis

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ABSTRACT

Adding poor solvent for polymer in the polymer solution will lead to shift in solubility and eventually phase separation, which refers to the solvent-shifting process. During this process it has been observed that relatively uniform size of polymer particles are formed. The formation of these polymeric nanoparticles have been assumed to follow a well-known “Ouzo Effect” that was previously used to explain the formation of nanoparticles of small-molecule organic molecules. This is also referred to as homogeneous nucleation. This approach has been used by many research groups to prepare particles of different sizes using polymers such as poly (lactic acid), polystyrene, and poly (methyl methacrylate). However, the assumed homogeneous nucleation mechanism for the solvent-shifting process has not been proved with obvious evidences.

In this research we have studied very dilute concentration of non-solvent to capture the early stages of concentration fluctuation and the formation of nucleation by dynamic light scattering. Small increasing of the scattering light together with 1~3 nm peak were observed at the early stage as adding water drop wise into PMMA/THF solution before PMMA nanoparticles were formed. Hypothesis and questions of the mechanism of the formation of polymer nanoparticle were carefully discussed in this article.
DEDICATION

This Master thesis is dedicated to my advisor, my group, my family and all my friends and colleagues who have provided me help and support during the research process.
ACKNOWLEDGEMENTS

I would like to thank my adviser, Dr. Ali Dhinojwala, for providing me the opportunity to conduct this project and helping me a lot with the experiments and knowledge, and thank Dr. Abraham Joy to be the committee member and reader. I also would like to thank all the group members in Dr. Ali dhinojwala’s group for their help with my experiment and helpful discussion of this project. Special thanks to Dr. Bojie Wang for my training in SEM and thanks to Dr. Tianbo Liu’s group for helping in DLS technique. Finally, I would like to thank all my friends and my families for supporting me in everything.
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CHAPTER I

INTRODUCTION AND BACKGROUND

1 Introduction of polymer nanoparticles

Nanoparticle, also termed as ultrafine particles, is defined as particles cover a range around one to hundred nanometers at around 1970-80’s.\textsuperscript{8} Regarded as a bridge between bulk materials and atomic or molecular structures, nanoparticles show significant change of properties as their sizes changes when approaches the nano-scale, which is different from bulk materials as the bulk ones have constant physical properties regardless of their sizes. The interesting and sometimes unexpected properties of nanoparticles involving optical properties, magnetic properties and surface properties has attracted research interest for decades.\textsuperscript{8,9}

1.1 Types of polymer nanoparticles and their structures

Nanoparticles can be fabricated by both inorganic and organic materials. The field of polymer nanoparticles (POLYMER NANOPARTICLE) has many unique properties which inorganic particles cannot approach.\textsuperscript{8} Polymer nanoparticle is a
collective term given for any kind of polymer nanoparticle, but specifically for nanospheres and nanocapsules. Nanospheres are matrix particles, of which the entire mass is solid and molecules may get adsorbed at the surface of the particles. Nanocapsules are vesicular systems with a hollow structure, inside the particle there is a liquid core (either oil or water) surrounded by solid material shell. Different systems can be represented as the following Figure 1.

![Figure 1. Illustration of classification of polymer nano-particles nanospheres (A), nanocapsules containing oil (B) and water (C).](image)

1.2 The application of polymer nanoparticles

Due to the easily tunable functionality, polymer nanoparticle has been applied into many areas such as drug delivery, structure color, elastomer enhance and others. The major application for polymer nanoparticle nowadays is drug delivery. Micelles formed by block copolymers with amphiphilic character have been regarded as a powerful carrier of drugs due their high drug-loading capacity of the inner core and the unique disposition characteristics in the body. Core segregation from aqueous milieu is the direct driving force for micellization and proceeds through a combination of intermolecular forces, including hydrophobic interaction.
electrostatic interaction, metal complexation, and hydrogen bonding of constituent block copolymer. A variety of drugs with different characteristics such as genes and proteins can be incorporated into the core of micelles. By adjusting the core-forming segment of the copolymer, it can provide a sufficiently strong interaction with the drug molecules. The system of polymer micelles for drug delivery can be described as shown in Figure 2.

Figure 2. (A) Features of polymer micelles that are relevant for drug delivery, (B) Structure of drug-conjugated block copolymer using doxorubicin and poly(ethylene glycol)-poly(α, β-aspartic acid) as an example.
In the nature, some of the most vivid colors in the animal kingdom are created not by pigments, but by wavelength-selective scattering of light from nanostructures with variations in index of refraction on length-scales of the order of visible light.\textsuperscript{8} This phenomenon is called the structural color. In recent years, periodic biological structures have provided inspiration for many groups trying to make photonic materials. However, the isotropic structures of the nature’s alternative design are just at the beginning of exploring. Hallam et al. have been succeed in using biomimetic random structures to make ultrathin mineral coatings with brilliant white color, Takeoka et al. recently showed a wide range of colors with very little angle-dependence can be produced by microgel dispersions and Forster et al. even produced a serious films with the structural color varies from white to deep blue by monodisperse polystyrene spheres containing carbon black assembled on substrates.\textsuperscript{19}

Figure 3. Photograph of five drop-cast films of 226 and 265 nm PS spheres containing carbon black.\textsuperscript{20}

In addition to these, Polymer nanoparticles also can contribute to the elastomer reinforcement acting as reinforcing fillers. Traditional reinforcing fillers, such as carbon black and silica, are usually solid powders.\textsuperscript{21} Although they are very effective for reinforcement, the drawbacks such as the difficult in blending the
powdered fillers with the high-viscous rubbers and even the poor compatibility with rubber matrix that may cause an aggregation that will do harm to the performance of the rubber. These problems may be due to the strong filler-filler interactions and weak filler-rubber interactions. Because of these problems, reinforcing fillers with some distinguishing features are expected. Polymer nanoparticles, which have many different features such as a well-controlled interaction between rubbers and fillers, some chemical activity on the particle surface that can provide extra functions and even the low price, have been regarded as a hope for the development of some novel elastomer reinforcing agents. A good example, from Lu et al., uses polystyrene particles synthesized by emulsion polymerization and divinyl benzene was introduced into the polymerization system as a crosslinking agent to enhance the glass-transition temperature and stiffness of the particles to improve the performance of reinforcement. As a result, it exhibited an excellent reinforcement to both polar and nonpolar elastomeric matrix such as styrene butadiene rubber (SBR), nature butadiene rubber (NBR) and nature rubber (NR), however, the tensile strength got deteriorated somehow. A further study in this area is really necessary.

2 Solvent-shifting technique for producing polymer nanoparticles

Since the properties of nanoparticles affect the performance in application dramatically, a well-controlled and easily-approached fabrication is really necessary. Generally, two main strategies are employed for the preparation of polymer nanoparticles: the dispersion of preformed polymers and the polymerization.
The most important technique for synthesis monomers into nanoparticles is emulsion polymerization utilizing surfactant. It was first employed for producing synthetic rubbers from 1,3-butadiene and styrene and after a dramatic development emulsion polymerization has become the predominant processs for the commercial polymerizations of vinyl acetate, chloroprene, various acrylate copolymerizations and copolymerizations of butadiene with styrene and acrylonitrile.\textsuperscript{25} Emulsion polymerization is a kind of radical chain polymerization that the reaction takes place in the system with monomers, water, emulsifier and water-soluble initiators. The emulsifier, also termed surfactant, is a kind of molecules that have both hydrophilic and hydrophobic parts so that it can separate the monomers and water phase.\textsuperscript{26} The use of water as dispersion medium is environmentally friendly and also leads to less significant viscosity and thermal problems than in bulk polymerization. The system and the process of emulsion polymerization can be explained in the Figure 4.

First the monomers and emulsifiers are added into the water phase at the same time. Due to the hydrophilic and hydrophobic force, droplets of monomers surrounded by emulsifiers are formed as shown above. Then some of the monomers will diffuse into the micelles formed by emulsifiers. After initiators added into the system and diffused into the micelles, the initiators will react with the monomers and cause a radical chain polymerization. The polymerization process goes very quickly until the reaction ended by chain transferring or termination, or until all the monomers are consumed. In the end in the system left some latex particles formed by polymer chains with the size around tens to hundreds nanometer scale.\textsuperscript{24}
One of the advantages of emulsion polymerization is that polymer nanoparticles with monodispersed size can be obtained due to the easily-controlled process based on its physical state of the system. However, the particle size and the molecular weight are hard to control although the fact that both the diameter and the molecular weight increases with the increasing extent of polymerization is well known. And the impurities including monomers, oligomers and surfactants can be difficultly removed from these polymerized nanoparticles and may affect the physical properties of the polymer core, thus frustrating measurements of the effect of confinement on polymer nanoparticle properties.

Figure 4. Typical emulsion polymerization system.

2.1 Introduction to solvent-shifting technique of polymer

The first method developed to prepare polymer nanoparticle from a preformed polymer is solvent evaporation, also termed as polymer emulsification process.
The system includes a liquefied water-insoluble polymer phase dispersed in an aqueous liquid medium phase containing at least one nonionic, anionic or cationic oil-in-water functioning emulsifying agent. During the process emulsions are formulated and the emulsion is converted into droplets or particles of the polymer solution dispersed in water, after which the solvent may then be removed as by stripping or other method of removal.\(^\text{30}\) It is widely employed for preparing nanoparticles that two main strategies are being widely used to prepare nanoparticles for the formation of emulsions: the preparation of single-emulsions and double-emulsions. It usually uses high-speed homogenization followed by evaporation of the solvent or by continuous magnetic stirring at the room temperature. Then the solidified nanoparticles can be collected after ultracentrifugation and washed by distilled water to remove the surfactants.\(^\text{22}\) Although the solvent evaporation method can be a simple method for the preparation of polymer nanoparticle, it is a time consuming method and the sizes, as well as the morphology of particles, are hard to control. And the method generally yields average polymer particles with the sizes about 1\(\mu m\), which is too large for many application.\(^\text{29}\)

As these two techniques above, polymer nanoparticles can also be generated through the displacement of a solvent with a non-solvent, which is termed precipitation.\(^\text{31}\) It was first developed by Fessi et al. for preparation of polymer nanoparticle\(^\text{31}\) which is called the interfacial deposition of the polymer. It was a novel and simple procedure for the preparation of nanocapsules by interfacial deposition of a preformed polymer following displacement of a semi-polar solvent.
miscible with water from a lipophilic solution. As an example in Fessi’s paper, PLA and phospholipids were both dissolved in acetone at first, then small amount of benzyl-benzoate was added into the acetone solution. Then the resulting organic mixture was directly poured into water consequently followed by the formation of nanocapsules, the wall of which is mainly constituted by the PLA polymer while the oily core by the benzyl-benzoate solution and water-acetone mixture as the surrounded environment. Based on this vesicular colloidal particle nanocapsules model, many oil-soluble drug can be involved into the core of the nanocapsules and the complex can work as drug deliver without the disadvantages, including unstable or rapid leakage, which involved in old vesicle system.

![TEM images of PLA nanocapsules with benzyl-benzoate core](image)

Figure 5. TEM images of PLA nanocapsules with benzyl-benzoate core.31

Another method named as solvent-shifting is also a very simple process by which hydrophobic molecules was first dissolved in a good solvent, which is miscible with water, and then introduce non-solvent into the solution. Both solvent-shifting and interfacial deposition were believed that based on the “ouzo effect” which take
place in the metastable region between the binodal and spinodal curves in the phase diagram of these mixture (Figure 6). If the solvent, for example, oil, is immiscible with water then a nanocapsules will form by interfacial deposition of polymer at the interface of oil droplets and water and once the solvent, for example, acetone is miscible with water, solid spherical nanoparticle will formed by solvent-shifting.

Figure 6. Right triangle three-component phase diagram at constant temperature and pressure. The ouzo effect occurs for solutions brought from the on-phase region into the metastable region between the bimodal and spinodal curves.\textsuperscript{37}

Different kind of polymers, for intense, PLA\textsuperscript{34}, PS\textsuperscript{35}, PMMA and many others have been successfully made into nanoparticles by solvent-shifting experiment, including dialysis and dropping, as shown in Table 1.\textsuperscript{35}
Table 1. Overview of selected molecular properties and synthetic methods of the polymer samples and z-average mean diameters of the resulting nanoparticles prepared by nanoprecipitation applying two different methods.\textsuperscript{39}

<table>
<thead>
<tr>
<th>Sample</th>
<th>M\textsubscript{w} kg mol\textsuperscript{-1}</th>
<th>\textit{PDF}\textsuperscript{a}</th>
<th>Method A: Diallyl</th>
<th>Method B: Dropping</th>
<th>Method A: DMA-H\textsubscript{2}O</th>
<th>Method B: Ac-H\textsubscript{2}O</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Synthesis grade</td>
<td>\textit{d} nm\textsuperscript{b}</td>
<td>\textit{PDI}\textsuperscript{c}</td>
<td>\textit{d} nm\textsuperscript{b}</td>
<td>\textit{PDI}\textsuperscript{c}</td>
</tr>
<tr>
<td>PS-1</td>
<td>5.2</td>
<td>NMP</td>
<td>489</td>
<td>0.233</td>
<td>418</td>
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<td>PS-2</td>
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<td>0.193</td>
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<td>TG</td>
<td>335</td>
<td>0.199</td>
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\textsuperscript{a} Determined by means of GPC. \textsuperscript{b} Average values of three DLS measurements, see the ESI. \textsuperscript{c} Also same large, undefined aggregates. \textsuperscript{d} Intradisperse in organic solvents. \textsuperscript{e} Only large, undefined aggregates. \textsuperscript{f} PDI: polydispersity index (\textit{M\textsubscript{w}}/\textit{M\textsubscript{n}}). \textsupersity: \textit{M\textsubscript{w}}/\textit{M\textsubscript{n}} polydispersity index of the particle size as determined by GPC. NMP: N-methyl-2-pyrrolidone polymerization. RAFT: reversible addition-fragmentation chain transfer. ATRP: atom-transfer radical polymerization. TG: technical grade.

Among them PLA was the most important polymer that has been made into nanoparticle by solvent-shifting due to its special contribution in the drug delivery area. The procedure always consists in the dissolution of PLA polymer and a specified quantity of the drug in organic solvent and then the organic phase was added drop wise into the aqueous phase under stirring until the complete evaporation of the organic solvent takes place. In the end PLA nanoparticle suspension was obtained.

Solvent-shifting method has been widely used in making PLA nanoparticles due to many advantages which are necessary for drug delivery. One of the most important one is that it can avoid many toxic impurities such as solvent, surfactant, initiator or small monomers which always been remained to the end by emulsion polymerization or solvent evaporation. Another point is that low energy consumption can be reached in the process of solvent-shifting. No heating is necessary and compared with other method solvent shifting is much less time consuming. In spite of this, the principle limitation of solvent shifting method is
about the problem for entrap water-soluble drug molecules, however, it still provided an easy method to produce drug delivery nanoparticles.

Polystyrene has also been tested with many different setup designed for solvent shifting method to produce nanoparticles and one of those different ways is called dialysis. First polystyrene will be dissolved in organic solvent, for example, acetone, and the polymer solution will be placed in the dialysis bag and dialysis against water. Dialysis bags are made by membranes with small holes that can let small molecules go through while large molecules will be blocked so that the polystyrene molecules will stay in the bag while acetone molecules were exchanged by water molecules due to osmotic pressure caused by the concentration gradient. As the fraction of water in the co-solvent increases while the fraction of acetone decreases in the dialysis process the polystyrene will precipitated out to form polystyrene nanoparticle. After keeping the dialysis for a long time that all the acetone is exchanged by water, polystyrene suspension in water can be obtained. 

Figure 7. Diagram of the dialysis setup
Another technique called Flash nano-Precipitation (FNP) mixing process was also developed to make polystyrene nanoparticle as shown in the Figure 8.\textsuperscript{39} It achieves rapid solvent displacement by means of novel high intensity mixing geometries that the mixing occurs in a central cavity fed by two incoming solvent steams (polystyrene solution and water) and as a consequence, sub-150 nm diameter particles of narrow size-distributions can be formed with high reproducibility, which is much smaller compared with the ones greater than 200 nm produced by a more time-consuming dialysis nanoprecipitation technique.\textsuperscript{39} And the size of the formed polystyrene nanoparticle has a strong relationship with the initial concentration of polystyrene solution (Figure 9).

Figure 8. Diagram of flash nano-precipitation (FNP) mixing process\textsuperscript{39}
Among those polymers, PMMA has been chosen as the most example to study the kinetic process of solvent-shifting and how different factors including concentration, temperature and pH and so on affects the formation of PMMA nanoparticles. Aubry and Ganachaud et al. successfully made PMMA nanoparticles with the diameter 100~500 nm.\textsuperscript{41} Both acetone and THF were chosen to dissolve PMMA and Brij56 was added into water before solvent shifting so as to help stabilize the formed PMMA nanoparticles as surfactant. Firstly PMMA was dissolved in organic solvent with different mass fraction varies from $10^{-5}$ to $10^{-2}$, then different amount of water dissolved with Brij56 was poured quickly into the PMMA solution with stirring and in the end PMMA nanoparticle can be formed as a nanoparticle suspension. Depending on the final fraction of the PMMA/organic solvent/water three component system, the size and morphology of the formed PMMA particle will be different whether the fraction stays in the “ouzo region” or not (Figure 10).\textsuperscript{41}
Figure 10. SEM images of PMMA nanoparticles formed (a) in the “ouzo region” or (b) beyond the “ouzo region” by pouring PMMA/acetone solution into water (with Brij56).\textsuperscript{41}

Schubert et al. prepared p(MMA-stat-MAA)\textsubscript{x:y} into nanoparticles with different size scale.\textsuperscript{42} In order to provide the possibility of functionalization for the nanoparticle carboxylic acid groups were introduced into the PMMA chain by copolymerizing MMA and MAA using RAFT polymerization method.\textsuperscript{43,44} Thus after the nanoparticles formed it can be further modified into different surface properties. By variation of the initial recipe of the formulation such as the solvent/non-solvent ration and the initial concentration of the polymer solution, the nanoparticle particles with different sizes can be prepared (Figure 11). Small (d<100 nm) and medium (d=100–200 nm) particles were produced by dropping polymer solution into water with the concentration at 1mg/ml and 10 mg/ml separately while large ones (d>300 nm) were produced by adding water into polymer solution with the concentration at 4mg/ml. In the end the acetone were evaporated out of the system so as to get polymer nanoparticle suspension in water.
They also successfully produced p(MMA-stat-MAA)$_{x:y}$ nanoparticles by dialysis 4 mg/ml PMMA/DMS solution against water with a similar dialysis setup as shown previously in Figure 7. DMA was used to dissolve the polymer and then the solution was dialyzed against water. By kept changing the surrounding water it was made sure that all the DMA was exchanged by water. Compare the formed nanoparticle from dialysis method and also from dropping with all other factors the same differences in size and morphology were found based on Figure 12.
In these studies, PMMA nanoparticle produced by different procedures as talked above varied with different conditions showed that different factors including viscosity, properties of polymer and solvent, and even molecular weight affects the size and morphology of the produced nanoparticles. In the experiment designed from Pietsch et al, the nanoparticles were produced by adding 12 different initial polymer solutions with concentration corresponding to the range of $c[\eta] = 0.004 \sim 0.120$ into eight different proportions of water in a way that the solvent/non-solvent ratio was ranged from 0.1 to 0.5 representing in total 96 different populations of nanoparticles as shown in Figure 13. A significant trend in following the changes of the parameters in the process can be observed that at the lowest initial concentration smaller nanoparticle formed compared with high concentration that after increasing the concentration the size increased from 70 nm to 180 nm. However, the solvent/non-solvent ration didn’t show an effect on the size distribution.
Figure 13. 3D representation of the formed nanoparticles by PMMA with $M_w = 7,700$ g/mol: an average particle size as function of initial concentration of polymer solution and solvent/non-solvent ratio.  

The solvent used in the experiment also make a huge difference in the size distribution while the molecular weight of the polymer seemed have no effect on the size of the formed particle based on the Figure 14. All the particles in Figure 14 were produced in acetone and THF, respectively, at a degree of dilution $c[\eta] = 0.01$ and a solvent/non-solvent ratio of 0.1 followed by evaporation of solvent. The sizes of the particle of PMMA 1,3 and 5 (prepared in acetone) were the same ($74 \pm 4$ nm) while the particle of PMMA 2,4 and 6 (prepared in THF) were same with each other ($100 \pm 20$ nm). However, as the molecular weight of PMMA in acetone increases the particles are no longer uniform and less spherically shaped while in contrast
particles prepared from THF are always well-confined spherical particles with PMMAs which have different molecular weight.

Although different kind of polymers have been successfully produced into nanoparticles by solvent shifting method with different setups, the way that different factors in the kinetic process affecting the final product is complex that a universal theory should be brought out to help explain phenomenon that occurs. However, when talking about the basic mechanism of the formation polymer nanoparticles it is always assumed that homogeneous nucleation and growth, which is the one for the formation of nanoparticles from small inorganic and small organic molecular, that taking place when the system was shifted into ouzo region (metastable area between the binodal and spinodal curve)
2.2 Homogeneous Nucleation and Growth

The process of nucleation occurs in the interior of a uniform substance is called homogeneous nucleation and the creation of a nucleus implies the formation of an interface at the boundaries of a new phase. The driving force of a homogeneous nucleation comes from the supersaturation of one component in the mixture by changing temperature, concentration or some other factors as shown in Figure 15.

![Phase diagram of a two component system](image)

Figure 15. Phase diagram of a two component system

The change of the free energy in the homogeneous nucleation process can be divided into two part, the increase in free energy caused from the newly created interface between the liquid and newly formed solid phase, $\Delta G_{\text{surface}}$, and the energy
released by creating a new volume, $\Delta G_{\text{volume}}$. Only if the balance based on positive $\Delta G_{\text{surface}}$ and the negative $\Delta G_{\text{volume}}$ can make the total change of the free energy $\Delta G_{\text{total}} = \Delta G_{\text{surface}} + \Delta G_{\text{volume}} < 0$ the process can be favored. If the hypothetical nuclei (unstable nuclei) is too small that the energy released from the formation of new volume is not enough to support the formation of new interface the nucleation is not favored and a critical size of the nuclei is necessary. For example, in the process of forming classic spherical nuclei, $\Delta G_{\text{total}} = \Delta G_{\text{surface}} + \Delta G_{\text{volume}} = \frac{4}{3}\pi r^3 \Delta G_v + 4\pi r^2 \sigma$ where $\Delta G_v$ is the change in the free energy per volume and $\sigma$ is the surface tension of the new interface. Since a negative total free energy is necessary to support the process a critical size (critical radius $r^*$) can be obtain as $r > \frac{-2\sigma}{\Delta G_v}$. After this critical point, the growth of the nuclei is no longer limited by nucleation but mostly by diffusion of the free molecules which is affected by many kinetic factors.

Based on the homogeneous nucleation and growth process, procedures for making inorganic nanoparticles have been designed and used in industry for decades. One of the earliest and most important application is the formation of sulfur nanoparticle. It was produced by a slow decomposition of dilute sodium thiosulfate in dilute hydrochloric acid. $2HS_2O_3^- = 2HSO_3^- + S_2$ is the reaction used to produce $S_2$ in the solution that sulfur may molecularly dissolved with a very low concentration. The formation of sulfur nanoparticle followed a step as shown in Figure 16 that it can be briefly divided into 3 stage. In the stage 1 the concentration of sulfur kept increasing as the reaction goes while no nuclei formed at that stage only
if after it reached a critical point. On reaching the metastable by making the sulfur supersaturated in the solution and once the supersaturation condition is able to overcome the energy barrier existed in forming nuclei the process will goes into the stage 2 where the rate of nucleation \((R_N) > 0\). Since the concentration of the sulfur in the solution was on a dynamic equilibrium balanced by the rate of the reaction and the rate of the formation of sulfur nuclei, once the reaction was slowed the supersaturation was relieved. After the Concentration went lower than the critical point, the nucleation stopped, however, since the sulfur was still supersaturated time dissolved sulfur molecules kept diffusing to the surface of the sulfur particle so that the growth of the sulfur nanoparticle was controlled by the diffusion in stage 3.

Figure 16. Schematic representation of the concentration of molecularly dissolved sulfur before and after nucleation as a function of time.
If the time for stage 2 was controlled very short, the number of nuclei will be fixed after stage 2 and the size only depended on the time of growth so that monodispersed sulfur particles can be formed and the driving force in this process was the change of sulfur concentration.

Not only for inorganic materials but also for organic small molecule the homogeneous nucleation have been used to produce organic nanoparticles. Several kinds of dyes, for example, the yellow cyanophenyl furanone dye, have been successfully produced into nanoparticle (Table 2).
Table 2. Organic Solvents Used in the Solvent-shifting Experiments

<table>
<thead>
<tr>
<th>solvent</th>
<th>mol wt</th>
<th>bp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-methylpyrrolidinone (NMP)</td>
<td>99.1</td>
<td>202.0</td>
</tr>
<tr>
<td>acetonitrile (ACN)</td>
<td>41.1</td>
<td>81.6</td>
</tr>
<tr>
<td>methyl sulfoxide (DMSO)</td>
<td>78.1</td>
<td>189.0</td>
</tr>
<tr>
<td>dimethylacetamide (DMA)</td>
<td>87.1</td>
<td>165.0</td>
</tr>
<tr>
<td>methanol (MeOH)</td>
<td>32.0</td>
<td>64.5</td>
</tr>
</tbody>
</table>

In this table, log\(P\) is the octanol/water partition coefficient whose values were obtained from the literature as cited\(^{48,49,52}\).

In those experiment the dye molecules were dissolved in a water-miscible organic solvent with high concentration and then the dye solution was directly poured into water and mixed by stirrer in the batch reactor as shown in the Figure 18.

Figure 18. Diagram of the precipitation batch reactor\(^{52}\)
Figure 19. SEM image of D-1 particles produced from (a) N-methylpyrrolidone (NMP) and (b) dimethylacetamide (DMA). Since the dye molecules were highly insoluble in water a supersaturation condition was created and the rapid precipitation of organic solute was favored to produce different dye nanoparticle based on different solvents that were used in the experiment (Figure 19, (a) and (b)).

Figure 20. Change of the diagram of the system before and after adding the dye solution into water.
The driving force in this process should be due to the change of the solubility parameter of the solvent that it changed the diagram of the system and leaded to a supersaturated condition as shown in Figure 11. Before added into the water the dye solution stayed in the stable area that the system remained homogeneous. After pouring the dye solution into water the organic will perfectly mixed with water to form the co-solvent for dye molecules. The mix of organic solvent and water will lead to a different solubility parameter for the co-solvent, which is more different from the one of dye molecules that the system will be driven from the stable situation into metastable between binodal and spinodal line, or even into unstable area.

2.3 Short summary for the solvent shifting technique for polymer nanoparticle

As a summary, the solvent shifting technique has become a very easy but powerful method to produce inorganic, organic and polymer nanoparticles. Several different kind of setups including dropping technique, dialysis method, and flash nano-precipitation and so on has been designed to help control the solvent shifting process. It becomes more and more widely used due the advantages, of which low energy cost and high purity are the most important, that it is hard for an emulsion polymerization to approach.

Many different kinds of polymer, such as polystyrene, PLA, and PMMA and so on have been successfully made into nanoparticles by solvent shifting technique and the products have been used in many different areas, especially in drug delivery system. As many factors including temperature, initial concentration of polymer
solution, viscosity, solvents and even different setups of solvent shifting will affect the kinetic process of forming polymer nanoparticle, it is not easy to control the size, morphology and, especially, the size distribution of the polymer nanoparticles. Thus a universal mechanism is necessary to help understand the whole story in the solvent shifting process, however, only an assumption that a similar one to the homogeneous nucleation and growth of inorganic and organic small molecule was made to the formation of polymer nanoparticles in the decade. Although a lot of information of the final particle products have been collected, no attention on the procedure of the formation and growth of the particles has been paid. Thus further research of learning the basic detail of the mechanism on this area is necessary.

3 Analysis methods of precipitation technique

The most important properties of polymer nanoparticles are morphology, size and size distribution. The morphology of the polymer nanoparticle is very important to its physical properties. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are important methods that can be used to determine the morphology of nanoparticles.

However, since the nanoparticles need to be taken out from the suspension and dried that the size and morphology may be changed during the procedure of preparation of SEM/TEM sample, it is important to test the nanoparticle properties in the original suspension. Dynamic light scattering is a very powerful technique that can be used to detect particles with a size range at 0.1–1000 nm. Dynamic light
scattering (DLS) measures the intensity/intensity-time correlation function \( G^{(2)}(\Gamma) \) by a multichannel digital correlation.\(^{50}\)

\[
G^{(2)}(\Gamma) = A(1 + b|g^{(1)}(\Gamma)|^2)
\]

In the correlation function, \( A \) describes the background, \( b \) refers to the coherence factor, and the \(|g^{(1)}(\Gamma)|\) refers to the normalized electric field time correlation function. The field correlation function can be analyzed by the constrained regularized CONTIN method to yield information on the distribution of the characteristic line width \( \Gamma \) from the following function.

\[
|g^{(1)}(\Gamma)| = \int G(\Gamma)e^{-\Gamma \tau}d\Gamma
\]

The obtained characteristic line width can be used to calculate an average apparent diffusion coefficient \( D_{\text{app}} \) from

\[
D_{\text{app}} = \frac{\Gamma}{q^2}
\]

Since the apparent hydrodynamic radius \( R_{h,\text{app}} \) is related to \( D_{\text{app}} \) based on the Stoke-Einstein equation

\[
D_{\text{app}} = \frac{kT}{6\pi\eta R_{h,\text{app}}}
\]

Where \( k \) is the Boltzmann constant and \( \eta \) is the viscosity of solvent at temperature \( T \). Thus from the DLS measurements, we can obtain the particle size distribution in solution from a plot of \( \Gamma G(\Gamma) \) verse \( R_{h,\text{app}} \), with \( \Gamma_i G(\Gamma_i) \) being proportional to the scattered intensity of all the \( i \) particles having an apparent hydrodynamic radius \( R_{h,i} \).
CHAPTER II

EXPERIMENTS AND PROCEDURES

1 Materials and equipment

Materials: Distilled water (Millipak® Sterile Millipore with 50 nm filter), THF (Sigma Aldrich reagent grade > 99.5%), PMMA (Scientific Polymer Products Approx. Mw=75,000), poly (n-butyl, α-(hydroxymethyl) acrylate) synthesized by Prof. Abraham Joy’s group (Mw=18000, it will be named as J-polymer for convenience), acetone (Fisher Scientific, certified ACS)

Equipment: Disposable Scintillation Vials(20 ml, Brookhaven Instrument Corporation, Holtsville, NY), Pasteur pipettes (9”, Flint Glass/Non-Sterile, Thermo Fisher Scientific Inc., Pittsburgh, PA), glass beakers (50~1000 ml, Thermo Fisher Scientific Inc. Pittsburgh, PA), Erlenmeyer flask (50 ml), glass syringe (0.5ml, 5ml, ), Nylon filter (450 nm, EMD Millipore Corporation, Billerica, MA), dialysis membrane and clips, vacuum oven (Fisher ISOTEMP® Model 281, Thermo Fisher Scientific Inc. Pittsburgh, PA), ultrasonic (Model 1510R-MTH, Branson ULtrasonics, Danbury, CT), dynamic light scattering (DLS, BI-200SM goniometer, version 2.0, Brookhaven...
Instruments Corporation, Holtsville, NY), Scanning Electron Microscopy (SEM, University of Akron).

2 Methods and procedures

2.1 Preparation of J-polymer nanoparticle

In this project, both dialysis method and dropping technique were used to prepare J-polymer nanoparticle. The structure of the J-polymer was shown in following Figure 21.

![Figure 21. Structure of the repeating unit of poly (n-butyl, α-(hydroxymethyl) acrylate)](image)

For dialysis method, at first polymer was weighted by balance and dissolved into 10 ml acetone to prepare a series of polymer solution with different initial concentration as shown in Table 3. 5 h magnetic stirring was applied so as to make sure the J-polymer was totally dissolved by the acetone. 450 nm nylon filter was applied to remove the dust in the solution and 3 times of the filter process were done before the solution was used. Distilled and deionized water was prepared by the Millipak® Sterile Millipore with 50 nm filter before use. Then a 1 L beaker with super distilled water and dialysis bag was prepared. Before using, the dialysis bag was immersed in the water to make it soft so as to open it, then it was washed by pure
acetone to remove all the water and wait a moment until all the acetone was evaporated. Thus the initial concentration of the polymer solution won’t be changed. Then the prepared polymer solution was poured into the dialysis bag that it is necessary to make sure the bag was just filled around 1/3 of its volume as it will swell with water diffusing into it. The bag was immersed into water and the dialysis process was kept under 25 °C with stirring. At the first 2 hours, the dialysis solution was replaced with fresh distilled water every 30 min. Subsequently, for the next 4 hours, the water was replaced every hour. The dialysis precipitation was allowed to take place over night and after 30 hours it was regarded as totally dialyzed. In the end the J-polymer nanoparticle suspension was removed into a 20 ml vial.

Table 3. Initial concentrations of J-polymer/acetone solution for dialysis

<table>
<thead>
<tr>
<th>No.</th>
<th>Initial concentration C_i (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.094</td>
</tr>
<tr>
<td>2</td>
<td>0.142</td>
</tr>
<tr>
<td>3</td>
<td>0.134</td>
</tr>
<tr>
<td>4</td>
<td>0.150</td>
</tr>
<tr>
<td>5</td>
<td>0.299</td>
</tr>
<tr>
<td>6</td>
<td>0.125</td>
</tr>
<tr>
<td>7</td>
<td>0.155</td>
</tr>
<tr>
<td>8</td>
<td>0.301</td>
</tr>
<tr>
<td>9</td>
<td>0.403</td>
</tr>
</tbody>
</table>
Table 3. Initial concentrations of J-polymer/acetone solution for dialysis (continued)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.204</td>
</tr>
<tr>
<td>11</td>
<td>0.663</td>
</tr>
<tr>
<td>12</td>
<td>0.800</td>
</tr>
<tr>
<td>13</td>
<td>0.514</td>
</tr>
<tr>
<td>14</td>
<td>1.005</td>
</tr>
</tbody>
</table>

For dropping technique, at first J-polymer was weighted by the analytical balance and dissolved into acetone to prepare the polymer solution with the initial concentration $C_i = 0.2 \text{mg/ml}$. 5 h magnetic stirring was applied so as to make sure the J-polymer was totally dissolved by the acetone. 450 nm nylon filter was applied to remove the dust in the solution and 3 times of the filter process were done before the solution was used. Distilled and deionized water was prepared by the Millipak® Sterile Millipore with 50 nm filter before use. All the vials, glass syringes, glass beakers and glass flasks were washed by acetone and Millipore water, after which followed by dried by nitrogen gas to avoid dust.

2.2 Preparation of PMMA nanoparticle

In this project, only dropping technique was used to prepare PMMA nanoparticles. PMMA was bought from Scientific Polymer with $M_w = 75,000$ with the structure as shown in the following Figure 21.
At first 5.5 mg polymer was weighted by the analytical balance and dissolved into 62 ml THF to prepare the polymer solution with the initial concentration $C_i = 1 \times 10^{-4}$ g/g. 5 h magnetic stirring was applied so as to make sure the PMMA was totally dissolved by the THF. 450 nm nylon filter was applied to remove the dust in the solution and 3 times of the filter process were done before the solution was used. Distilled and deionized water was prepared by the Millipak® Sterile Millipore with 50 nm filter before use. All the vials, glass syringes, glass beakers and glass flasks were washed by acetone and Millipore water, after which followed by dried by nitrogen gas to avoid dust. 15 vials (20 ml) with 6 ml PMMA/THF solution were set and water with different volume was added into the vial based on the following Table 4.

Table 4. Volume of PMMA/THF and water added in different vials

<table>
<thead>
<tr>
<th>No.</th>
<th>Volume of solution (ml)</th>
<th>Volume of water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 4. Volume of PMMA/THF and water added in different vials (continued)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>6</td>
<td>1.6</td>
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</tr>
<tr>
<td>5</td>
<td>6</td>
<td>1.7</td>
<td></td>
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<tr>
<td>6</td>
<td>6</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
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<td>10</td>
<td>6</td>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>2.4</td>
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<tr>
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<td>6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>3.5</td>
<td></td>
</tr>
</tbody>
</table>

Every vial was shaken by hand to make it well mixed and was put under dynamic light scattering (DLS) to test the scattering intensity change upon time. After the scattering intensity of the mixture was stable the DLS data was recorded.

2.3 SEM and Dynamic light scattering

The hydrodynamic radius and the size distribution were determined by DLS after the sample was kept in the 20 ml vial. Samples were detected under the temperature at 25°C with the scattering angle at 90 degree. Laser source was
stabilized for 5 minutes until the intensity of the light was stable. 20 ml vial with particle suspension was placed in the sample cell and the pump was run for 3 minutes to filter the solvent in the sample cell so as to avoid the error caused by the dust in the sample cell. After confirming that the laser went through the vial from the center the detector was turned on to collect data.

Figure 23. Diagram of the Dynamic Light Scattering (DLS) system

First of all, scattering intensity of the sample can be determined by the BI-200SM goniometer in the DLS instrument that can be used to determine the turbidity of the mixture. Then, autocorrelation function was recorded by the software provided by the Brookhaven Instruments Corporation. The autocorrelation function was then be calculated by CONTIN so as to get the information of a plot of $\Gamma G(\Gamma)$ verse $R_{\text{h,app}}$. 
The morphology of the polymer nanoparticle was determined by SEM, and the sample needs to be specially prepared for SEM. After the polymer nanoparticles were formed, the nanoparticle suspension was dropped onto the stub that was used for SEM test. Silica substrate was cut into 1 cm × 1 cm square and was attached to the stub by conductive type. Then the modified stub with nanoparticle suspension droplet on it was put into the vacuum oven and dried under the vacuum condition at room temperature for 1 hour to remove all the solvent and water, which was required for SEM experiment. In the end, the sample was coated by gold so as to improve the conductivity of the sample to increase the resolution. Then the SEM image of the polymer nanoparticle was recorded by the SEM instrument following the guideline.

Figure 24. Diagram of the SEM sample of polymer nanoparticle
CHAPTER III

RESULTS AND DISCUSSION

1.1 Morphology of J-polymer nanoparticle products determined by SEM

The morphology of J-polymer particle products from dialysis method and dropping technique is shown in Figure 24. These images illustrate that smooth J-polymer particles can be successfully generated using dialysis method and dropping technique, which comparing with previous research on solvent-shifting is a similar result. While the SEM can be only used to prove the morphology of the nanoparticles formed by dropping due to the change of the size during the evaporation of solvent during the preparation of SEM sample, for nanoparticles formed by dialysis no further growth will happen because all the solvent has already been removed so that more information can be obtained from the SEM image. As the initial polymer concentration increases in the dialysis membrane, an increase in diameter of nanoparticle produced by dialysis method and a broadening of nanoparticle size distribution are observed qualitatively in Figure 25. Bigger sphere nanoparticles can
be observed with initial concentration $C_i = 0.403$ mg/ml than others while it is obvious that the distribution of the size of nanoparticles will be larger when $C_i = 0.403$ mg/ml as there’re many small particles along with those big ones. The increase in both diameter and size distribution can be explained using the mechanism of nucleation-aggregation alluded to earlier. It was believed that that the probability for a nucleus to encounter another nucleus or aggregated nuclei increases, resulting in larger nanoparticle sizes, and the nuclei randomly aggregate suggests that the size distribution should broaden with nanoparticle size. However some results from DLS show a much more complex phenomenon that will be discussed later in the PMMA experiment.
Figure 25. (A) SEM image of J-polymer particle produced by dialysis method with initial concentration $C_i = 0.155$ mg/ml. (B) SEM image of J-polymer particle produced by dropping technique with initial concentration $C_i = 0.2$ mg/ml and volume fraction of water is $V_w\% = 20\%$.

Figure 26. (A) SEM image of J-polymer particle produced by dialysis method with initial concentration $C_i = 0.094$ mg/ml. (B) SEM image of J-polymer particle with initial concentration $C_i = 0.134$ mg/ml. (C) SEM image of J-polymer particle with initial concentration $C_i = 0.155$ mg/ml. (D) SEM image of J-polymer particle with initial concentration $C_i = 0.403$ mg/ml.
As shown above most of the nanoparticles are sphere, however, some defects are found from the SEM images shown in Figure 26. As at the top left corner of Figure 26 (A) some nanoparticles with hallows, generated by dialysis method, were observed. And in Figure 27 (B-D), in which nanoparticles are generated by dropping technique, when the volume fraction of water is only $V_{w\%} = 10\%$ (B) a lot of particles with hallow can be found when the amount of water increases to $V_{w\%} = 20\%$ (C). The defects begin to decrease and even almost disappear when the volume fraction of water reaches around $V_{w\%} = 30\%$ (D).

![Figure 27](image)

Figure 27. (A) SEM image of J-polymer particle produced by dialysis method with initial concentration $C_i = 0.142$ mg/ml. (B) SEM image of J-polymer particle generated by dropping technique with volume fraction of water around $V_{w\%} = 10\%$. 
(C) SEM image of J-polymer particle generated by dropping technique with volume fraction of water around $V_w \% = 20 \%$ (D) SEM image of J-polymer particle generated by dropping technique with volume fraction of water around $V_w \% = 30 \%$

Compared with previous SEM results of PMMA, PS, or even PLA nanoparticles produced by solvent-shifting process, those spherical structures are similar with each other while no such hollow structure was observed before. This interesting structure remains to be proved by further cryo-TEM experiment.

1.2 Size and size distribution of J-polymer nanoparticle products from dynamic light scattering (DLS)

From the Dynamic Light Scattering (DLS), intensity-time correlation function was provided for samples with different concentrations.

a. Results of dialysis methods

In Figure 28 it is the correlation function for the sample with initial concentration $C_i = 0.301 \text{ mg/ml.}$

![Figure 28](image.png)

Figure 28. Intensity-time correlation function of the J-polymer nanoparticle product sample with initial concentration $C_i = 0.301 \text{ mg/ml.}$
The correlation function was analyzed by the constrained regularized CONTIN method, using WinCONTIN software, to yield the information of the size and the size distribution of the nanoparticle products. In Figure 29 it is the size and the size distribution information for the sample with initial concentration $C_i = 0.301$ mg/ml.

Figure 29. The size and size distribution of the nanoparticle product sample with the initial concentration $C_i = 0.301$ mg/ml

A series of samples of J-polymer nanoparticle were made by dialysis method with different initial concentrations and the data calculated as shown above are listed in Table 5.

Table 5. DLS data of sample produced by dialysis method with different initial concentration

<table>
<thead>
<tr>
<th>No.</th>
<th>Initial concentration $C_i$ (mg/ml)</th>
<th>Mean radius R (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.094</td>
<td>66</td>
</tr>
<tr>
<td>2</td>
<td>0.142</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>0.134</td>
<td>78</td>
</tr>
</tbody>
</table>
Table 5. DLS data of sample produced by dialysis method with different initial concentration (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Concentration</th>
<th>Radius (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.150</td>
<td>129</td>
</tr>
<tr>
<td>5</td>
<td>0.299</td>
<td>138</td>
</tr>
<tr>
<td>6</td>
<td>0.125</td>
<td>138</td>
</tr>
<tr>
<td>7</td>
<td>0.155</td>
<td>145</td>
</tr>
<tr>
<td>8</td>
<td>0.301</td>
<td>151</td>
</tr>
<tr>
<td>9</td>
<td>0.403</td>
<td>152</td>
</tr>
<tr>
<td>10</td>
<td>0.204</td>
<td>156</td>
</tr>
<tr>
<td>11</td>
<td>0.663</td>
<td>159</td>
</tr>
<tr>
<td>12</td>
<td>0.800</td>
<td>163</td>
</tr>
<tr>
<td>13</td>
<td>0.514</td>
<td>165</td>
</tr>
<tr>
<td>14</td>
<td>1.005</td>
<td>176</td>
</tr>
</tbody>
</table>

Figure 30. Curve of radius as a function of initial concentration of polymer in acetone with bars showing distribution of the sizes.
The curve of radius as a function of initial concentration is shown in Figure 30 and the non-linear fit is applied by Origin Pro 8. Here from Figure 30 it becomes clear that with an increase of initial concentration of polymer solution, after remaining at a small size a dramatic increase of the size of nanoparticle will occur and then the size will turn into a constant number around $R = 160$ nm. The critical point is around $C = 0.2$ mg/ml to $C = 0.3$ mg/ml that after that the size of the nanoparticle will stay the same no matter the change of the initial concentration. This shows a relationship exist between the size of the nanoparticle and the initial concentration, and the maximum of the radius of these nanoparticles produced by dialysis method is only around $R = 160$ nm to $R = 170$ nm. What’s more, the bar in the figure shows the distribution of the size of the PNP products that all the samples have a wide distribution of the size.

b. Results of dropping technique

With dropping technique J-polymner nanoparticles are produced by dropping water into the polymer solution to different volume fraction at the initial concentration of water in acetone $C_i = 0.2$ mg/ml. The volume fractions of water combined with the size of the nanoparticle respectively are listed in Table 6 calculated based on the same procedure as in the dialysis method section.
Table 6. Volume fraction of water and the size of nanoparticle produced by dropping technique

<table>
<thead>
<tr>
<th>No.</th>
<th>Volume fraction of water $V_w%$</th>
<th>Mean radius R(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.8</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>7.7</td>
<td>67</td>
</tr>
<tr>
<td>3</td>
<td>13.7</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>19.4</td>
<td>110</td>
</tr>
<tr>
<td>5</td>
<td>21.9</td>
<td>135</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>259</td>
</tr>
<tr>
<td>7</td>
<td>33.3</td>
<td>337</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>387</td>
</tr>
<tr>
<td>9</td>
<td>45.5</td>
<td>408</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>429</td>
</tr>
</tbody>
</table>

The curve of radius of nanoparticles as a function of volume fraction of water is drawn as Figure 31 and the non-linear fitting is applied by Origin Pro 8.
Figure 31. Radius of nanoparticles a function of volume fraction of water prepared using the dropping technique with bars showing distribution of the sizes.

From Figure 31 it is able to figure out that the polymers dissolved in acetone will precipitated at a very low volume fraction of water and the initial radius is around \( R = 60 \text{ nm} \). Then the radius of the nanoparticle increase slightly from \( R = 60 \text{ nm} \) to \( R = 100 \text{ nm} \) until the volume fraction of water reaches around \( V\% = 25\% \) that the radius increases dramatically to over \( R = 400 \text{ nm} \) and remains steady again.

The change of the radius of the particle during the dropping technique is quite different from that in dialysis process that it can be divided into three stages while the dialysis method only has two. At the meanwhile, the maximum radius of nanoparticle products produced by dropping technique is around \( R = 400 \text{ nm} \) to \( R = 450 \text{ nm} \), which is much larger than that obtained by dialysis methods. And by using dialysis method, polymer solution with initial concentration \( C_i = 0.2 \text{ mg/ml} \) in acetone can only generate nanoparticle products with radius \( R = 165 \text{ nm} \). As mentioned before, the mechanism of the dialysis method and the dropping technique are assumed to be the same by pervious research, however, based on the DLS data of both dialysis method and dropping technique, they show very different results that a quiet different process during these two methods can be predicted. These differences may be caused by the differences between the kinetic process between dropping and dialysis diffusion but further evidences are necessary to explain it. However, with dropping technique it was able to observe the detail process during the formation of polymer
nanoparticles besides the final result, dropping experiment with much more precise process needs to be done. PMMA, which has a lot of research results can be used to be compared with, was used in this project to study the formation details.

2.1 Morphology of PMMA nanoparticle products determined by SEM

The particles in Figure 32 was produced by adding water into the PMMA/THF solution with the initial concentration of \( C_i = 1 \times 10^{-4} \text{g/g} \) followed by evaporating all the water and THF. Spherical PMMA nanoparticles were formed as shown in the SEM image that matches precious study. As the water percent increased the size of the particles also increased with two size distributions shown up, one with the radius around 100 nm while another with the radius around 500~700 nm. This two size distributions phenomenon was also observed in the DLS experiment. However, the sample for SEM test need to meet the requirement of solid state that no solvent should exist. Thus a further of the growth of the formed particles will take place during the evaporation of the THF and the size and morphology of the PMMA particle should be changed so that the SEM can only be used to determine the morphology of the PMMA particles without the size information. Thus a technique that can be used to monitor the in situ information of the PMMA particle, dynamic light scattering, is necessary in this project for PMMA/THF/water system.
Figure 32. SEM image of the PMMA nanoparticle produced by adding water into PMMA/THF solution with the initial concentration $C_i = 1 \times 10^{-4} \text{ g/g}$. (a) mass percent of water $w\% = 30.15\%$; (b) mass percent of water $w\% = 31.05\%$; (c) and (d) mass percent of water $w\% = 32.79\%$.

2.2 Scattering intensity and size distribution information of PMMA nanoparticles from DLS

Size information of the PMMA nanoparticles formed by dropping method was tested and recorded by the Dynamic Light Scattering (DLS). Data of PMMA/THF/water mixture with different volume of water added were recorded separately. Scattering intensity of the sample can be read from the record of the
software. Table 7 shows scattering intensity data of PMMA/THF/water mixtures.

Table 7. Scattering intensity of PMMA/THF/water mixture with different volume of water added. (Initial concentration of PMMA/THF solution is $C_i = 1 \times 10^{-4} \text{g/g}$)

<table>
<thead>
<tr>
<th>No.</th>
<th>Volume of solution (ml)</th>
<th>Volume of water (ml)</th>
<th>Mass Percent of water (%)</th>
<th>Scattering Intensity (kcps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.00</td>
<td>0.00</td>
<td>0</td>
<td>24.8±0.3</td>
</tr>
<tr>
<td>2</td>
<td>6.00</td>
<td>1.00</td>
<td>15.80</td>
<td>26.0±0.2</td>
</tr>
<tr>
<td>3</td>
<td>6.00</td>
<td>1.50</td>
<td>21.97</td>
<td>38.8±3.1</td>
</tr>
<tr>
<td>4</td>
<td>6.00</td>
<td>1.60</td>
<td>23.09</td>
<td>42.7±5.4</td>
</tr>
<tr>
<td>5</td>
<td>6.00</td>
<td>1.70</td>
<td>24.19</td>
<td>46.5±7.1</td>
</tr>
<tr>
<td>6</td>
<td>6.00</td>
<td>1.80</td>
<td>25.25</td>
<td>50.4±7.7</td>
</tr>
<tr>
<td>7</td>
<td>6.00</td>
<td>1.90</td>
<td>26.29</td>
<td>54.7±10.2</td>
</tr>
<tr>
<td>8</td>
<td>6.00</td>
<td>2.00</td>
<td>27.29</td>
<td>57.8±9.8</td>
</tr>
<tr>
<td>9</td>
<td>6.00</td>
<td>2.10</td>
<td>28.27</td>
<td>62.1±8.2</td>
</tr>
<tr>
<td>10</td>
<td>6.00</td>
<td>2.30</td>
<td>30.15</td>
<td>124.3±9.1</td>
</tr>
<tr>
<td>11</td>
<td>6.00</td>
<td>2.40</td>
<td>31.06</td>
<td>235.3±19.4</td>
</tr>
<tr>
<td>12</td>
<td>6.00</td>
<td>2.60</td>
<td>32.79</td>
<td>315.9±23.9</td>
</tr>
<tr>
<td>13</td>
<td>6.00</td>
<td>2.80</td>
<td>34.45</td>
<td>822.6±97.3</td>
</tr>
<tr>
<td>14</td>
<td>6.00</td>
<td>3.00</td>
<td>36.02</td>
<td>982.7±49.4</td>
</tr>
<tr>
<td>15</td>
<td>6.00</td>
<td>3.50</td>
<td>39.65</td>
<td>1179.0±35.1</td>
</tr>
</tbody>
</table>
Figure 33. Change of the scattering intensity following the adding of water into the PMMA/THF solution with initial concentration $C_i = 1 \times 10^{-4} \text{g/g}$

By plotting scattering intensity verse the mass percent of the water into the PMMA/THF solution with the initial concentration $C_i = 1 \times 10^{-4} \text{g/g}$, the process can be roughly divided into 3 region as shown in Figure 33. In Region 1, from the beginning when there was no water added into PMMA/THF solution the scattering intensity was very low (around 25 kcps) and it kept at that low level even if water was kept adding into the solution. Then after entered into Region 2, the slope of the curve became increasing that the scattering intensity start to increase slowly from 25 kcps to 60 kcps. And the scattering intensity dramatically increased with a much larger slope after adding water from Region 2 into Region 3. The scattered light intensity relates to many different factors such as the number of particles, the size of particles and also the reflective index and so on. Thus also the increase of the intensity can be a signal of formation of particles, more detail information on size is
necessary. Every point on the curve of Figure 33 refers to one sample with or without different PMMA nanoparticle that the autocorrelation function was recorded and was transferred into a plot of $\Gamma G(\Gamma)$ verse $R_{h,\text{app}}$ by CONTIN software telling the size distribution of the obtained PMMA nanoparticle as shown in the following Figure 34 (a ~ l).

Started from the point that water with the mass fraction of 23.09% was added into the PMMA/THF solution particles started to form with the size range of 1~10 nm as shown in the Figure 34 (a). Since the size was only around 1~10 nm, without any SEM images to prove a particle morphology and previously there are no report about polymer nanoparticles formed by solvent-shifting at this size scale, it is very hard to tell the peak refers to 1~10 nm PMMA nanoparticles. However, with more and more water was added into the PMMA/THF solution, the size distribution of the formed particle changed. Based on the summary as shown in Figure 34 (l) PMMA nanoparticles with different size distributions were formed which can be roughly divided into three kinds, small particle (1~10 nm), intermediate particle (10~100 nm) and large particle (>100 nm).
(a) Size distribution of PMMA particles formed by adding 23.09% water

(b) Size distribution of PMMA particles formed by adding 24.18% water
(c) Size distribution of PMMA particles formed by adding 25.25% water

(d) Size distribution of PMMA particles formed by adding 26.28% water
(e) Size distribution of PMMA particles formed by adding 27.29% water

![Graph showing size distribution of PMMA particles formed by adding 27.29% water.]

(f) Size distribution of PMMA particles formed by adding 28.27% water

![Graph showing size distribution of PMMA particles formed by adding 28.27% water.]

54
(g) Size distribution of PMMA particles formed by adding 30.15% water

(h) Size distribution of PMMA particles formed by adding 31.06% water
(i) Size distribution of PMMA particles formed by adding 32.79% water

(j) Size distribution of PMMA particles formed by adding 36.02% water
(k) Size distribution of PMMA particles formed by adding 39.65% water

(l) Summary of size distribution of PMMA nanoparticle produced with different amount of water added.
Figure 34. (a ~ k) Size distribution of the PMMA nanoparticle produced by adding different amount of water into PMMA/THF solution; (l) Summary of the size distribution from a to k.

At the beginning of adding water into the PMMA/THF solution, when the scattering intensity slightly increased there were only small unknown particles formed and no intermediate and large particles came out. Then after further dropping of water a second peak referred to the intermediate nanoparticles showed up as shown in Figure 34 (f). Then a third peak of large particles was formed following the dropping of more and more water into the PMMA/THF solution. The intermediate particles matches the small particles observed in SEM images on size while the large particles matches the large one in the SEM image. The size change of these three different kinds of PMMA particles can be plotted as Figure 35.
Figure 35. The change of the size of the small, intermediate and large PMMA particles as the different amount of water was added into the PMMA/THF solution.

It is obvious that the small unknown particle formed at first with the size only around 1~10 nm. Then the intermediate ones showed up, following by which immediately the large particles formed. And during the process the unknown small particles always existed and it is the same story for intermediate PMMA particles after they were formed, which tells that both of them were keeping forming in the process. Based on the consequence that these three kind of nanoparticles formation the conclusion that the large PMMA particles were formed from intermediate particles while the intermediate ones were formed based on the small ones.

However, there are two different possibilities of the formation from smaller ones into big ones. The first possibility is that the free chains dissolved in the THF/water co-solvent slowly condensed on the interface of the formed smaller PMMA nanoparticle and the solvent during the process of adding water. As more and more water was added into the mixture, THF/water co-solvent’s Hildebrand solubility parameter shifted from THF’s solubility parameter, $\delta=9.52\text{ cal/cm}^3$, to one with the number much closer to water’s solubility parameter, $\delta=23.4\text{ cal/cm}^3$. The Hildebrand solubility parameter for PMMA was $\delta=9.3\text{ cal/cm}^3$, which is close to the one for THF, it can be dissolved in THF very well with the concentration $C_i = 1 \times 10^{-4}\text{ g/g}$. After more and more water was added into the system drop by drop, as the solubility of co-solvent became larger and large and after reach a critical point the system shifted from the stable area into metastable area and with a continuous
dropping of water supersaturation was reached so that PMMA was not able to dissolve in the co-solvent anymore and precipitated out to form 1~10 nm small nanoparticle. After the concentration of PMMA in THF decreased so that the system became stable again the formation of small nanoparticle ends. With more and more water added into the system, however, the free chains of PMMA was not be able to be dissolved in co-solvent anymore so that they diffused to the surface of the PMMA nanoparticle and condensed to form new surface and the DLS data shows to peaks. And as more and more PMMA chains condensed on the surface making the PMMA particle grew bigger and bigger the large particles were formed with a third peak showed up in the DLS data.

However, the existence of the three kinds of PMMA particles without transitions between each other indicates a different mechanism of the growth of the PMMA particle that it might be possible that the intermediate PMMA particles were formed by combination of small ones while the large ones came from the combination of intermediate ones. But now there is no answer for the question that how a smooth surface of the large particles can be formed from the combination of small ones. Another important issue is that by detecting the zeta potential of the formed PMMA nanoparticles it showed a negative charge on the surface the particles, which can be a huge barrier that needed to be overcame before the small particles collapse into one large particle.
Figure 36. Zeta potential diagram of the PMMA nanoparticle formed by adding water with the mass percent of 36.02% into PMMA/THF solution with the initial concentration $C_i = 1 \times 10^{-4} g/g$

Beyond the process of the formation and growth of the PMMA particle, the size of the unknown small particles, which is 1~10 nm, is also very interesting. If a simple calculation is applied based on the following equation to a single PMMA chain with a molecular weight $M_w = 75,000$ the size of the PMMA nanoparticle formed by only one polymer chain can be solved out.

$$\frac{M_w}{N_A \times \rho} = \frac{4}{3} \pi R^3$$

In this equation, the assumption that solid glass spherical PMMA particle is formed. $M_w$ is the molecular weight of the polymer chain, $N_A$ is the Avogadro Constant, $\rho$ is the density of the formed PMMA particle which is assumed to be 1.2 g/ml (provided by Scientific Polymer Product Inc.) and $R$ is the radius of the formed PMMA particle. Thus the radius can be calculated as:
\[ \frac{3 \times M_w}{4 \times \pi \times N_A \times \rho} = R^3 \]

\[ R \approx 2.92 \text{ nm} \]

Compared with the calculated size of the single chain particle, the small PMMA nanoparticle with the size of 1~10 nm obtained by dropping water into the PMMA/THF shows a size with only one or two chains contained inside. As the small ones appeared at the first step of the formation of PMMA particles that the nucleation process is supported that free chains condensed into solid coil from free chains to act as nuclei for further growth. However, as DLS can tell light scattering from any component that the unknown small ones may also be some other diffusion at the 1~10 nm size scale. To make a conclusion that it is solid PMMA nanoparticle at 1~10 nm now without further evidence such as cryo-TEM image is not proper.

\[ R \approx 2.92 \text{ nm} \]

Figure 37. Potential structure of the 1~10 nm single chain small PMMA particle

In a summary, the formation of PMMA was proved by both SEM image and DLS experiment while the growth of the PMMA particle was observed by DLS experiment. The morphology of the formed PMMA particles matches the observed
phenomenon by previous research. A relationship between the size and the amount of water that was added into the system was observed. Compared with the formation J-polymer by dropping technique, the trends of the size change verse the added water are similar with each other and a more detail information can be obtain from PMMA/THF/water system based on the DLS data we obtained. Two hypothesis was made to explain the process of the formation and growth of the PMMA nanoparticle while more evidences were necessary to make a final conclusion on this phenomenon. And an unknown particle at 1~10 nm scale remains a secret to be proved.
CHAPTER IV

CONCLUSION

In summary, both J-polymer was successfully made into particles by both dialysis method and dropping method. Smooth spherical structure was obtained and it matches previous study on solvent-shifting method for making polymer nanoparticles. Also a relationship between the size distribution and the initial concentration and also the amount of water added was founded. In order to get much more detail information and make it possible to compare with previous data, PMMA, which is a common polymer used before, was used to make into nanoparticles with THF as solvent and water as non-solvent. In this project, by adding water drop wise into the system with very small cutoff, the formation of the PMMA nanoparticle was proved by both SEM image and DLS while the growth of the PMMA particles was observed and studied based on the DLS experiment. During the process of adding water into the PMMA/THF solution, at first no PMMA particles were formed before critical point was reached. After 23.09% water was added into the solution some unknown small PMMA nanoparticles with the size of
1~10 nm was formed at first and larger PMMA particles, in this project we call it intermediate particles with the size of 10~100 nm, were formed following by kept adding 30.15% water into the system. Finally large PMMA particles was formed starting from the point that 32.79% water was added.

The formation of the PMMA nanoparticle was assumed to be favored by the supersaturation condition caused by adding water into the system to create a worse solvent for PMMA. After the solubility parameter increased from the one of THF which is $\delta=9.52 \text{ cal/cm}^3$ to a higher one that close to $\delta=23.4 \text{ cal/cm}^3$ for water the PMMA chains followed a homogeneous nucleation process to form nuclei by the condensation of few PMMA chains. Then a growth of the PMMA particle occurs with adding more and more water into the system.

Two hypothesizes has been made to explain the growth of the PMMA particles. The first one assumes that it was the condensation of the free PMMA chain on the surface of the small particles due to the shift of the solubility parameter of the THF/water co-solvent caused the growth of the PMMA particles. But it failed to explain the discontinuous of the size distribution of the PMMA particles. Another hypothesis assumes that the growth of the PMMA particles was caused by the combination of the smaller particles. However, the negative charged surface of the PMMA particles is a big challenge for the collapse of these particles that make this hypothesis work. Based on this situation, further evidence is necessary so as to help distinguish the mechanism of the formation and growth of the polymer nanoparticles in the solvent-shifting process. Another important point is the unknown 1~10 nm
particle formed at the early stage in the dropping process that no enough evidence right now can be used to prove a condensed PMMA chain structure. Also it seems that they provided a start point of the growth of the particle, it can be anything with a diffusion at that size scale so that further evidences such as cryo-TEM are necessary in the future.
CHAPTER V

FUTURE WORK

In the future there are several work need to be done so as to prove the hypothesis made in the project. First of all, cryo-TEM images of the PMMA particles with different sizes need to be taken, especially for the unknown 1~10 nm particles to prove the structure. Method that can be used to separate the particles out of the system without changing the morphology and size of them is a huge challenge in the future that by overcoming it many different applications can be developed. Also PMMA with different solvent, different solvent-shifting rate, different concentration and many other different factors should also be tried to compare the formation process. And by comparing different polymer’s solvent-shifting process such as PS, PLA, PMMA that were studied before or J-polymer that were specially synthesized by ourselves, a university rule was hoped to be pointed out.
REFERENCES


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