CREATING MULTI-COMPONENT NANOPARTICLES USING NANOPRECIPITATION

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By

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ABSTRACT

Nanoprecipitation is a self-assembly process for forming nanoparticles by replacement of a good solvent in which multiple materials are dissolved with a poor solvent. It has demonstrated its advantages of easy setup, good size controllability for single component nanoparticles formation, the potential for mass production and the ability of being customized to form different kinds of composite nanoparticles. This research focuses on exploring the capabilities of the nanoprecipitation method for forming complex nanoparticles and attempting to gain a better understanding of the formation. Two distinct multi-component nanoparticle systems were selected as model systems: “immiscible blends of polystyrene (PS) and poly (methyl methacrylate) (PMMA)” and “perylene-tetracyanoquinodimethane (TCNQ) molecular co-crystals”. The nanoprecipitation processes were carried out with a typical stirred vessel set up for fast mixing and the systems were brought into a high supersaturation condition for the concentrations and solvent ratios used. This work demonstrates the formation of PSPMMA immiscible blend nanoparticles with a core-shell structure, while aggregation and network formation of PMMA was observed. A model taking into account the glass transition temperature (Tg) confinement effect is proposed in explaining the differences between PSPMMA blend samples and control group samples. This work reveals the importance of the nature of the polymers in the blend, especially the glass transition, on the behavior of the final
products. Research on perylene-TCNQ co-crystal nanoprecipitation was a continuation of earlier work done by Nishida. The formation of 3:1 stoichiometry nano co-crystals (P3T1) by nanoprecipitation was demonstrated for an initial perylene/TCNQ ratio of 3:1, as its crystal structure was confirmed by powder X-ray diffraction (PXRD) spectrum. The finding was further supported by absorption spectra, dynamic light scattering (DLS) data and scanning electron microscopy (SEM) images. For an initial perylene:TCNQ ratio of 1:1, the resulting sample contained not just the 1:1 stoichiometry nano-crystals (P1T1), but also the P3T1 crystals as well as nanocrystals of pure TCNQ and possibly perylene. This work shows that the initial stoichiometry of materials is not the only factor dictating the final product of co-crystal nanoprecipitation, although the detailed mechanism is not yet well understood based on current experimental data.
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1 INTRODUCTION

This work aims to investigate the multi-component nanoparticle system using a specific synthesis method, the ‘Nanoprecipitation’ method. Before a detailed description of the research progress and results, the relevant basics of multi-component nanoparticle systems and background on the nanoprecipitation method are provided in this introduction.

1.1 Multiple-component Nanoparticles

After decades of development, nano science and technology remains of interest in both academia and industry. Many different kinds of nano systems are studied for various applications. Novel nanomaterial development has always been one of the major trends within this area. The power of ‘nano’ lies in the quantum mechanical effects and effects induced by large surface/volume ratio. These effects emerge when the particle size is reduced to length scales in the range of 100’s nm and below, and they markedly change the properties of the materials from those of bulk state, including mechanical, biochemical, optical and electrical properties (Horn, Rieger 2001). For instance, the solubility of organic materials in water significantly increases in nano form comparing to that in bulk form (Horn, Rieger 2001). In the case of gold, its nanoparticle can appear to be ruby red instead of the golden color, and if the size is small enough, a metal-insulator transition will occur (Daniel, Astruc 2004). Similarly, silver nanoparticles, as potential inorganic antimicrobial agents, have larger contact surface area with microorganisms and
are considered to be more effective than traditional Ag ions and salts (Kim, Kuk et al. 2007).

With the rising need to deal with more complex cases, however, the limitation of the single functionalities of single component nanoparticles is revealed. In the face of new challenges, more and more attention is being paid to synthesizing multi-component nanoparticles and this area of research is gaining huge momentum in developing novel nanomaterials. Multi-component nanoparticles may possess combined properties from the individual components or even new properties arising from the interaction between the components. These unique properties can help multi-component nanoparticles surpass some of the limits of single component materials and lead to potential applications in areas of pharmaceuticals, coatings, and microelectronics. The biomedical and pharmaceutical area is one of the major research territories for composite nanoparticles, and a recent review given by Singh illustrates some research achievements of multifunctional materials in drug delivery systems. In drug delivery research, multifunctional nanoparticles are usually in the form of nanocapsules and nanospheres (e.g. biopolymers and nanomembranes) within which drug or pharmaceutical agents are captured. These multifunctional nanoparticles are designed to carry and deliver specific drugs to targeted sites and also to help control drug release. They have shown to have many advantages over traditional drugs for future application and bring hope for treating severe diseases such as cancer (Singh, Lillard Jr 2009). In other areas, breakthroughs also have taken place for potential applications. Nanosized charge transfer co-crystals, for example, could have promising electronic and photovoltaic properties that might be useful in organic solar cells (Nishida 2011).
Many studies of multi-component nanoparticles are still in the fundamental research stage and have not yet reached industrial application. The major challenge is how to synthesize these materials in a predetermined way with precise control while meeting the requirements for mass production. Most of the time, to overcome this challenge, scientists either start with techniques with precise control ability and then improve the techniques to achieve mass production, or vice versa. Molecular self-assembly processes have the potential of mass production of nanoparticles since molecular building blocks spontaneously aggregate during the process and form nanoparticles or structures as a result of interactions between molecules and their surroundings. No obvious external driving force is present in the process. By applying the molecular self-assembly with proper design of molecular structures or functional groups of the original building blocks, complicated nano-systems with different architectures or functions are highly achievable in a simple and feasible fashion (Ouellette 2000); and plenty of research breakthroughs have been reported in labs and research centers (Lu, Sastry 2007). The nanoprecipitation technique used in this research is a method of molecular self-assembly which shares the fundamentals and advantages of molecular self-assembly. Efforts are being undertaking to apply this method to multi-component nanoparticle formation and in achieving precise control of the final products (Schubert, Delaney Jr et al. 2011).

1.2 Nanoprecipitation Technique

1.2.1 Introduction of Nanoprecipitation

Nanoprecipitation is a process of spontaneous emulsification of a solute due to a change of the solvent power of the dispersing medium (e.g. a polar substance in a nonpolar
solution). In a typical nanoprecipitation process, a molecular solution is first made by dissolving a molecular species into a good solvent. The molecular solution is then mixed with a miscible non-solvent to achieve a reduction in solvency power of the solution. A miscible non-solvent is a solvent that is miscible with the good solvent but in which the molecular species has poor solubility. Due to the sudden drop of solubility and resulting supersaturation after the mixing, the spontaneous aggregation of the molecular building blocks in the solution follows (Kasai, Nalwa et al. 1992, Van Keuren, Nishida 2010). Nanoprecipitation has different nomenclatures in the publications, which is referred as, but not limited to the names of, “miscible solvent shifting” (Texter 2001), “solvent displacement” (Beck-Broichsitter, Rytting et al. 2010), “Ouzo process” (Beck-Broichsitter, Rytting et al. 2010, Aubry, Ganachaud et al. 2009), “reprecipitation” (Horn, Rieger 2001) and “Flash nanoprecipitation” (Zhang, Pansare et al. 2012, Sosa, Liu et al. 2016). Also, there are different variations of the nanoprecipitation technique, such as “self-organized precipitation” (Yabu 2013), “super critical fluid methods” and “salting out”, depending on the ways of modifying the solvent solubility power (Texter 2001).

The concept of nanoprecipitation has existed for a long time. A Greek drink called “Ouzo” is an ancient example of nanoprecipitation. The drink turns into milky white when water is added into anethole oil dispersed alcohol and causes formation of micron-sized anethole droplets (Botet 2012, Ganachaud, Katz 2005). The same concept has later been utilized and patented since the 1800’s to fabricate polymeric end products that are used in paints and as drug excipients, or as an intermediate process to refine and purify polymers (Schubert, Delaney Jr et al. 2011). LaMer and his coworkers have reinvestigated similar concept to form nanosized inorganic hydrosols in the 1940s
(LaMer, Dinegar 1950, Reiss 1951). In recent decades, Horn and coworkers in BASF applied a nanoprecipitation method in a \( \beta - \text{carotene} \) hydrosol production in late 80s (Horn 1989, Horn, Rieger 2001). Later, Fessi et al. patented the idea of forming polymeric nanodispersions using nanoprecipitation (Fessi, Puisieux et al. 1989, Fessi, Devissaguet et al. 1992). In late 90s, the Nakanishi group at Tohoku University published a series of paper on organic nanocrystals by nanoprecipitation (Kasai, Oikawa et al. 1998, Sarkar, Okada et al. 2000) followed by other groups producing optical materials including various dyes (Kim, Bjorklund et al. 2003, Keuren, Georgieva et al. 2001, Keuren, Georgieva et al. 2003). The momentum has continued in recent years: nanoprecipitation has been intensively studied as a way to capture other species into polymer nanoparticles or to form composite nanoparticles with various morphologies. Examples that have been reported in the literature include: drug delivery nanoparticles (Yang, Hua et al. 2009, Chiellini, Chiellini et al. 2001, Schubert, Delaney Jr et al. 2011), polyplexes from oppositely charged molecules (Duncan, Ringsdorf et al. 2006, Schubert, Delaney Jr et al. 2011), Janus or multi-facet nanoparticles (Sosa, Liu et al. 2016, Yabu 2013), charge transfer nano co-crystals (Nishida 2011), etc.

The nanoprecipitation method has many advantages compared to other methods. It is easy to implement due to its simple synthesis setup and procedure. It is very versatile and is able to produce nanoparticles in the form of nanocapsules, nanospheres, liposomes, vesicles, etc. (Bilati, Allémann et al. 2005, Ganachaud, Katz 2005, Whittemore IV, Jones et al. 2015). Its capability in independent size control has also been demonstrated within single component nanoparticle systems and is promising to expand to more complicated systems (Aubry, Ganachaud et al. 2009, Zhang, Pansare et al. 2012, Perevyazko, Vollrath
et al. 2012). In addition, the nanoprecipitation process does not demand high-energy input comparing to processes like grinding, ball milling, high-pressure homogenization or ultrasonication, which may cause material degradation, yield heterogeneous products, or cannot be used with unstable materials (Whittemore IV, Jones et al. 2015, Aubry, Ganachaud et al. 2009, Brick, Palmer et al. 2003). As shown in $\beta$ – carotene production, nanoprecipitation has the potential to be carried out in continuous manner for industrial scale mass production (Horn, Rieger 2001). It also carries the advantage of preparing material by precipitation processes and in dispersion form. For instance, it can be easier to transport and store certain nanoparticles in dispersion form. Meanwhile, challenges also exist. Low solute concentrations are often required for nanoprecipitation to yield homogeneous stable nanoparticle dispersions accurately and repeatedly, while it is usually harder to control the nanoparticle size distributions at higher concentrations (Vitale, Katz 2003, Aubry, Ganachaud et al. 2009, Brick, Palmer et al. 2003). In addition, the particle formation mechanism in nanoprecipitation is still not fully understood, especially the early formation stage (Horn, Rieger 2001). Moreover, the published work on nanoprecipitation varies widely in terms of set ups (mixing conditions), stabilization mechanisms (surfactants) and material used (eg. for polymers: different MW or polymer end groups; small organic molecules) and so on. This is a downside of easy customization and it requires researchers to be more careful when use other groups’ findings as research guidance. It also brings challenges in comparing one’s own research results to those of others, since numerous factors might affect the final nanoparticles (size or morph) and the actual formation mechanism.
1.2.2 Nanoprecipitation Set ups

![Schematic diagrams of stirred vessel set-ups](image)

**Figure 1.1 Schematic diagrams of stirred vessel set-ups.** Mixing by (a) injecting and (b) dropping molecular solution into miscible non-solvent.

Different set ups have been used to perform the nanoprecipitation in order to meet various needs and requirements. Here are listed a few commonly used ones. The stirred vessel, as shown in Figure 1.1, is one of the most frequently used set-ups for nanoprecipitation. The nanoprecipitation process can be accomplished by injecting or dropping the molecular solution (a mixture of solute and good solvent) with a syringe or pipette into a stirred non-solvent. Alternately, the non-solvent can be added to the solution. The injection or dropping process can be done manually, or sometimes it has even been simplified by pouring one solution into another, and the stirring is not always performed (Aubry, Ganachaud et al. 2009, Nishida 2011). These manual processes are dynamic and turbulent, and they have been shown to be good enough for qualitative experiments and for on-off testing of the sample formations or when the system is not very sensitive to the mixing condition. Manual nanoprecipitation is very simple to perform and requires low investment so that it is often used in laboratories (Schubert,
Delaney Jr et al. 2011). However, many systems are sensitive to the initial mixing condition, in which cases manual injection can cause bad sample reproducibility and lacks precise control over the properties of the final particles due to the arbitrary human errors when performing the mixing procedure. To overcome the drawbacks of the manual mixing, automatic injection or dropping can be done by using automated syringe pumps (as shown in Figure 1.2) or using a designed batch reactor or a pipetting robot.

Impinging jet mixers are another kind of set up used for nanoprecipitation. These can achieve rapid non-solvent displacement by high-intensity mixing, and have shown promising results for precise particle size control (Johnson, Prud'homme 2003b, Zhang, Pansare et al. 2012, Brick, Palmer et al. 2003). As shown in Figure 1.3 (a), two streams of flow (one stream is the molecular solution, another is the miscible non-solvent) from two inlets rapidly converge in the mixing container and then the mixed solution flows into a reservoir containing abundant miscible non-solvent. Besides the bulk nanoprecipitation set ups, micro platforms offer alternative options to perform nanoprecipitation. Figure 1.3 (b) and (c) present microfluidic platforms with slightly different micro-mixing structures, they are based on the hydrodynamic flow focusing mechanism to achieve predictable particle formation (Karnik, Gu et al. 2008, Capretto, Cheng et al. 2012, Valencia, Basto
et al. 2010). On small length scales of mixing, the particles are constrained in the central focused stream and form upon solvent diffusion. The inlet flows are automatically controlled by syringe pumps. Both the flow rates of the inlets and the width of the central focused stream can be separately adjusted to control particle formation. The particle formation can be further controlled by changing the angles and length of the mixing channels or by integrating the zigzag mixing ‘Tesla structure’ as shown in Figure 3(c).

With proper adjustment of the designs of the setups mentioned above, they can also be used in continuous flow mode in which steady state nanoprecipitation conditions can be achieved (also known as “Stop flow” devices). Characterization techniques such as DLS can be coupled with the stop flow device to monitor the nanoprecipitation process in real time.
2 NANOPRECIPITATION

2.1 Nanoprecipitation in General

2.1.1 Introduction and Scope of Discussion

Much of the research on the nanoprecipitation method has been experimental, with an emphasis on specific applications. Various reports in the literature have focused on investigating and tuning different factors to achieve desired nanoparticle size and properties. Fewer studies have been done on the fundamentals of nanoprecipitation, and most of these have applied phenomenological models (based on the deducing the possible mechanism of formation from the final product behavior) to single component nanoprecipitation (Horn, Rieger 2001). Nevertheless, the currently available theoretical models achieve qualitative or semi-quantitative agreement with experimental findings, although the understanding of nanoprecipitation is not yet comprehensive (Horn, Rieger 2001, Botet 2012).

This section focuses on the basics of the nanoprecipitation method and the key findings from the literature, including a discussion of the thermodynamics of nanoprecipitation in terms of the ternary phase diagram of solute/solvent/non-solvent, the factors affecting particle behavior and the proposed formation mechanisms. Detailed discussions on the formation mechanism are subdivided into polymer specific and small organic molecule specific parts, since these two material systems may be considerably different from each other even though they share some commonalities. Unless specified, the systems of discussion here limit to hydrophobic homopolymers (which contains only one type of repeating unit) and small organic molecules (also hydrophobic), which are the systems I
investigated experimentally. Copolymers or amphiphilic small molecules, which may have both hydrophobic and hydrophilic parts, behave differently and can be found elsewhere in the review literature.

2.1.2 Phase Diagram and Ouzo Region

2.1.2.1 Phase Diagram of Ternary System

Both thermodynamics and kinetics are relevant in the nanoprecipitation process. Ternary phase diagrams are often used as a starting point to understand the systems of single component nanoprecipitation thermodynamically, which can also lend insight to more complicated composite nanoprecipitation (e.g. quaternary systems).

A typical ternary phase diagram is shown in Figure 2.1(a) (Botet 2012) and Figure 2.2 (a) (Lai, Lin et al. 1998). The whole phase diagram is divided into several regions. The single-phase region is where the components are miscible with each other; while in the two-phase region, the system is no longer in thermodynamic equilibrium and the mixture becomes supersaturated. To reduce the total free energy of the system, the mixture phase separates into different phases along the tie lines, a solute rich phase and a solute poor (solvent rich) phase (Lai, Lin et al. 1998, Botet 2012). The line that separates these two regions is the binodal (miscibility) line, which is the equilibrium boundary of the system. The two-phase region is further divided into the metastable region and the unstable region by the spinodal (stability) line, depending on the phase separation details. In the metastable region, the system goes through a nucleation and growth mechanism by overcoming a certain energy barrier. In the unstable region, the system phase separates spontaneously by the spinodal decomposition mechanism. Figure 2.1(b) illustrates the
two-phase separation mechanisms in a binary system of polymers, in which the time scale of phase separation phenomenon is long enough to be observed (Bates 1991).

Figure 2.1 Schematic diagrams of ternary phase diagram and phase separation. (a) Schematic ternary phase diagram of solute/solvent/water system (Botet 2012), © IOP Publishing. Reproduced with permission. All rights reserved; (b) Phase separation by Nucleation and Growth mechanism and by Spinodal Decomposition mechanism is illustrated by polymer experiments for binary system. From (Bates 1991). Reprinted with permission from AAAS.

In the nanoprecipitation literature, a variation of the ternary phase diagram is also often used, with solute composition (in final mixture) as the horizontal axis and solvent composition (in final mixture) as the vertical axis (Aubry, Ganachaud et al. 2009, Brick, Palmer et al. 2003, Vitale, Katz 2003), as shown in Figure 2.2 (b). In these phase diagrams, the horizontal scale is logarithmic since nanoprecipitation is often carried out at very low solute fractions. During the nanoprecipitation, the system is brought from a stable, single-phase state (solvent phase with solute dissolved) to a supersaturated state by mixing in enough non-solvent. Complex structures form without external driving force as the system minimizing its free energy (Jones 2002). This nanoprecipitation pathway is schematically represented as the curve line in gray color in Figure 2.2 (b), which ends at the two-phase region. From the end point, the system follows the phase separation lines (known as tie lines) into solvent-rich and solvent-poor phases. Note that the phase
separation in nanoprecipitation leads to metastable colloidal systems, not the macroscopic phase separation in the thermodynamic sense. In real systems, the exact pathway can be different depending on the details of mixing and time scales of phase separation.

Figure 2.2 Two constructed ternary phase diagrams of polymer solutions. (a) Theoretically calculated binodal lines and tie lines: PMMA/n-butyle acetate/n-hexane system (solid line) and PMMA/Acetone/Water system (dash line), and these lines well match with experimental findings. Reproduced from (Lai, Lin et al. 1998) with permission of John Wiley & Sons; (b) phase diagram of PMMA/Acetone/Water system that is constructed based on experimental data and theoretical calculations: back symbols – experimental binodal line, symbols with different shapes relate to PMMA with different molecular weight and polydispersity; red symbols – experimental ouzo boundary; black and green lines are theoretically calculated binodal and spinodal lines. Reprinted (adapted) with permission from (Aubry, Ganachaud et al. 2009). Copyright (2009) American Chemical Society.

Phase diagrams can be valuable in initial experiment design of nanoprecipitation, such as the selection of materials to meet the miscibility requirements or considerations of concentrations to begin with. Phase diagrams also help clarify possible formation mechanisms, e.g., whether by nucleation and growth or by spinodal decomposition (Brick, Palmer et al. 2003). The phase diagram for a specific ternary system can be constructed from both experimental data and theoretical calculations. Experimentally, the binodal line is usually determined by titration methods such as cloud point measurement. Theoretically, the phase diagram can be estimated from the free energy of mixing $\Delta G_M$:

$$\Delta G_M = \Delta H_M - T \Delta S_M$$
in which $\Delta H_M$ is the enthalpy of mixing, $T$ is the temperature and $\Delta S_M$ is the entropy of mixing. It relates to interaction energies among the components and the entropy change and determines the miscibility and solubility. $\Delta G_M$ must be negative for materials to be miscible. Once the expression of $\Delta G_M$ is known, calculation of the binodal line, spinodal line and criticality can be carried out (Bates 1991).

Practically, it is challenging to get the exact expression of free energy of mixing of a complicated system like those in nanoprecipitation. A common approach is estimating the terms of free energy of mixing based on well-established theories of simple systems, such as the regular solution theory of binary systems developed in 1930’s by Hildebrand and Scatchard and Flory-Huggins theories for polymer-polymer or polymer solution systems (Hildebrand, Wood 1933, Scatchard 1931, Bates 1991). They capture the basics of the underlying physics and the essence of the phase separation phenomenon despite the simplifications that are made. For instance, the experimental observations on phase separation shown Figure 2.1(b) match well with the theoretical predictions based on the Flory-Huggins theory of polymer-polymer systems (Bates 1991). Modifications are made to expand those theories to ternary systems and to account for certain features of more complicated cases. Meanwhile, experimental efforts are needed to determine values of certain parameters used in the theoretical analysis and to help justify the end results. Figure 2.2 shows the work done by Lai et al. and Aubry et al. in constructing ternary phase diagrams of polymer/solvent/non-solvent systems (Lai, Lin et al. 1998, Aubry, Ganachaud et al. 2009). In their work, binodal lines constructed theoretically match with the experimentally determined lines. Tie lines and spinodal lines are theoretically calculated based the information obtained from binodal lines.
2.1.2.2 The Ouzo Region in Phase Diagrams

The ouzo region refers to a region of certain fractions of solute/solvent/non-solvent in which solute spontaneously forms into nanoparticles with good monodispersity. Outside of the ouzo region, nanoparticles and microparticles can coexist in the final dispersion or macroscopic phase separation takes place (Aubry, Ganachaud et al. 2009, Vitale, Katz 2003). The boundary of the ouzo region defines the maximum concentration of the solutes in a fixed solvent/non-solvent combination to form stable nanosuspensions.

The ouzo region is of great value in guiding the design of processing conditions and helping optimize the size control and drug loading (Beck-Broichsitter, Rytting et al. 2010, Schubert, Delaney Jr et al. 2011). It is determined experimentally. In most literature, the determination of the boundary is based on final particle size information or the ability to redisperse sediments of the dispersion after centrifugation. In a system of liquid nanoprecipitation, it is where the failure of accurate size measurement occurs due to rapid oil droplet coalescence at those ratios of component (Vitale, Katz 2003). For polymer nanoprecipitation, Aubry et al. used 1.2 µm pore size filter as a measure to separate macroparticles from nanoparticles, in other words, particles larger than 1.2 µm are considered macroparticles. The light scattering intensity of the particle dispersion after filtration was compared with the original unfiltered portion. If the system is beyond the ouzo boundary, separation of nanoparticle from macroparticle by filtration will cause a decrease in intensity (Aubry, Ganachaud et al. 2009). Another method used by Aubry et al. was checking the ability to redisperse sediments from dispersion after centrifugation. If the sediments are fully redispersable by a vortex, the corresponding condition is considered to be within the ouzo region. Outside of the ouzo region, sediments cannot be
redispersed (Aubry, Ganachaud et al. 2009). Both methods arrived at similar ouzo boundaries, although slightly different, and it was claimed that this finding supports the description of the ouzo phenomenon (that is, more macro particles present in the dispersion beyond the ouzo region).

2.1.2.3 Current Knowledge and Challenges

The ouzo region is currently not well understood theoretically. The challenge of developing a theoretical model of the ouzo region is difficult because nanoprecipitation is a process dependent on both thermodynamics and kinetics (Botet 2012). In a system of the same materials and ratios of components, the final particle size can vary dramatically for different mixing conditions. For instance, Brick et al. found better mixing by high-energy impinging jets can result smaller particles than by mixing in a stirred vessel (Brick, Palmer et al. 2003). Similar findings are widely observed in other work (Fessi, Devissaguet et al. 1992, Molpeceres, Guzman et al. 1996). In addition to the difficulties defining the ouzo region theoretically, it is possible to determine it experimentally only approximately. The arbitrariness comes from the experimental techniques used, the continuous feature of the system in the region near the ouzo boundary, the man-made criteria of the ouzo boundary and the variance of waiting time before size measurement among literature, as can be seen in the methods mentioned above. More specifically, the nanodispersion by nanoprecipitation is not perfectly monodisperse, and the sizes of nanoparticles change continuously with the ratios of components, even near the ouzo boundary. As a result, only arbitrary experimental definitions of the ouzo boundary are possible, such as choosing a certain pore size for filtration (e.g. 1.2 µm in work of Aubry et al.) Moreover, the waiting time after the nanoprecipitation for size measurement is also
relevant and the involvement of the solvent removal process also matters. Finally, the light scattering method and the centrifugation method have their limitations in size determination accuracy.

Usually, the ouzo region is located in the region of high supersaturation and low solute concentration, as found in most nanoprecipitation systems. Many researchers claim that the ouzo region is located within the metastable region (between binodal and spinodal line) in the phase diagram (Schubert, Delaney Jr et al. 2011, Vitale, Katz 2003, Aubry, Ganachaud et al. 2009). This might be the case for some particular systems as claimed in the work of Vitale or Aubry, but it is questionable to expand this claim to all nanoprecipitation systems due to the ambiguity of the ouzo limit just discussed and also because the lacking of knowledge of the exact spinodal line for complicated systems in nanoprecipitation. For instance, Brick et al. suggested that their nanoprecipitation system passed the spinodal line and reached the unstable region under certain conditions (Brick, Palmer et al. 2003).

2.1.3 Factors Influencing Nanoparticle Growth in Nanoprecipitation

Various important factors, both thermodynamic and kinetic, have been extensively studied in nanoprecipitation. Investigations have focused on the effect of factors on different aspects of nanoprecipitation, including: the feasibility of nanoparticle formation, e.g., the ability to produce supersaturation, stabilization mechanism, sufficiency of interdiffusion (Bilati, Allémann et al. 2005, Schubert, Delaney Jr et al. 2011), the location of the ouzo boundary, particle size and distribution, morphology of both single component and composite nanoparticles (spherical or irregular shape, amorphous or
crystalline) and phase separation within composite nanoparticles (structures: core-shell, Janus, multifacet or matrix), ingredient entrapment and efficiency, ingredient leakage and sample reproducibility, etc.

The typical way of studying a particular factor is isolating it by fixing other parameters. The frequently investigated factors include nanoprecipitation setup, concentrations and ratios of components, the nature of the materials, temperature etc. They are frequently used as controllable processing parameters (eg. size tuning) to form desired products. This section introduces the basics and examples of reported findings of selected factors in nanoprecipitation. Some key findings and examples will be revisited in detail later in specific sections.

2.1.3.1 Nature of Materials and Their Interactions

The nature of the components and their interactions (solute, good solvent and non-solvent) are essential in affecting particle behavior by determining material affinity and miscibility.

2.1.3.1.1 Nature of Solute

Different solute types can result in distinct particle morphology. For instance, nanoprecipitation of polymers usually results in amorphous nanoparticles; while for small organic molecule systems, both amorphous (Brick, Palmer et al. 2003) and crystalline (Keuren, Georgieva et al. 2003) nanoparticles have been reported. For composite polymer nanoprecipitation, using different polymer combinations can result in either core-shell or Janus particle structures (Motoyoshi, Tajima et al. 2010).
For polymer systems, changing the molecular weight or end groups of the polymer can lead to change in size and morphology. Prud’homme’s group found that varying the molecular weight of one polymer in polymer blend nanoprecipitation not only affects the size and distribution of blend nanoparticles but also changes the particle morphology from Janus structure to a multifaceted structure (Sosa, Liu et al. 2016). It has also been observed that the same polymer with and without ionizable end groups responds differently to the same solvent-nonsolvent combinations, forming nanodispersion with different particle size and distribution and stability (Bilati, Allémann et al. 2005, Roger, Eissa et al. 2013). The nature of the solutes can also affect the efficiency of entrapment of ingredients with low molecular weight. For instance, lipophilic drug ingredients are much better incorporated into hydrophobic polymeric nanoparticles than hydrophilic drug ingredients (Fessi, Devissaguet et al. 1992, Beck-Broichsitter, Rytting et al. 2010, Hyvönen, Peltonen et al. 2005).

2.1.3.1.2 Solvent/Non-solvent

Solvent and non-solvent have also been extensively studied and their influence has been confirmed on the success of forming particles with nanoprecipitation (ouzo limit), the particle size and distribution, the particle morphology and leakage of encapsulated drugs.

The choice of solvent of different types can cause size changes and affect the ouzo boundary. As observed in the works of Galindo et al. and Aubry et al., the solvent type affects the critical concentration (equivalent to the idea of the ouzo limit) as well as the particle size in polymer systems (water as non-solvent) (Galindo-Rodriguez, Allémann et al. 2004, Aubry, Ganachaud et al. 2009). But it is not always the case, as illustrated in
work of Beck-Broichsitters et al. comparing THF, acetone and acetonitrile as solvents in polymer nanoprecipitation (water as nonsolvent), in which final particles using acetone and acetonitrile have similar sizes while they are smaller than those obtained with THF (Beck-Broichsitter, Rytting et al. 2010). For different solvents in the same homologous chemical series, the final particle sizes also change, as illustrated in Bilati’s paper using ketones as solvent for polyester nanoprecipitation (Bilati, Allémann et al. 2005). Another set of experiments varying the solvent nature was done by incorporating small amounts of the non-solvent into the solvent before nanoprecipitation (Fessi, Devissaguet et al. 1992, Thioune, Fessi et al. 1997). It was found to help reduce polymer loss when the system is beyond the ouzo region. The solvent type can also affect the particle shape as shown in the work of Aubry: PMMA nanoparticles in THF/water system are less spherical than they are in acetone/water system (Aubry, Ganachaud et al. 2009).

For non-solvent, water is most frequently used in the literature, mainly due to the requirements of biocompatibility and non-toxicity in applications. Alcohols were also tested as an alternative to minimize hydrophilic drug leakage in study of forming drug carriers by nanoprecipitation (Bilati, Allémann et al. 2005). Moreover, it was found that changing non-solvent affects the final particle sizes, regardless of whether the non-solvents are from different material types or from the same homologous chemical series (Bilati, Allémann et al. 2005).
2.1.3.1.3 Dielectric Constants and Solubility Parameters

Attempts have been made in the literature to explain observed correlations between the nature of materials (and their interactions) and the particle behavior. The affinity between materials is considered essential to particle size since it largely affects the interdiffusion behavior of the components. A common belief is that sufficient interdiffusion between solvent and non-solvent (sufficient diffusion coefficient and low viscosity) is necessary for the success of nanoprecipitation. Most reports agree that enhancing the affinity of solvent with nonsolvent or reducing affinity between solute and solvent can help interdiffusion of solvent and non-solvent, which can lead to smaller particle size (Bilati, Allémann et al. 2005, Whittemore IV, Jones et al. 2015). Motoyoshi et al. also tried to justify the morphology of polymer blend nanoparticles by estimating the affinity of materials (Motoyoshi, Tajima et al. 2010). It can be challenging to determine the exact degree of affinity between materials, so parameters such as dielectric constant and solubility parameters are often used to estimate the interactions among the components and their affinity.

2.1.3.1.3.1 Dielectric Constants

The dielectric constant (more strictly, the relative static permittivity, notation $\varepsilon$) is commonly used to approximate the polarity of materials. Thioune et al. made an early attempt applying dielectric constants to help understand the relation between solvent nature and system behavior (Thioune, Fessi et al. 1997). In a hydrophobic polymer/acetone/water system, the author found that using a combination of acetone and a small amount of water as solvent helps reduce polymer loss beyond the ouzo region,
and more water incorporated in the solvent (less than a maximum percentage) leads to lower polymer loss. The author attributed the polymer loss reduction to the increasing value of the dielectric constant of the solvent when more water was added (or more properly, the smaller discrepancies of dielectric constants of solvent and non-solvent by adding one to the other). Note that incorporating non-solvent into the solvent phase changes the conformation of the polymer macromolecules before nanoprecipitation, so the starting point of nanoprecipitation in this case is located within the one-phase region, not on the solute-solvent axis. In later investigations within the ouzo region, the correlation of the dielectric constants (or the difference of dielectric constants) with particle size behavior is found to be very weak in most cases. A very broad and approximate trend, if any, is that the larger the differences of dielectric constants between solvent and non-solvent, the larger the particle size or more likely, the failure of nanoprecipitation (based on polymer systems) (Bilati, Allémann et al. 2005). In a few cases, good correlation was observed, usually in systems where either the solvent or the non-solvent was varied in a homologous chemical series (Bilati, Allémann et al. 2005).

The weak correlation between dielectric constants and particle size might be due to its limitation in reflecting affinity of materials in some specific systems. An example supporting this statement is the failure of dielectric constant in justifying the miscibility between THF and water: THF has very low value of dielectric constant ($\varepsilon = 7.52$), while water has a very high value ($\varepsilon = 80.1$). Based on values of dielectric constant alone, they should be immiscible. In reality, they are completely miscible due the hydrogen bonding. Despite the limitation of applying dielectric constants, they can be still used as a quick or preliminary study for picking solvent/non-solvent combinations for a particular solute,
since the dielectric constant is comparatively easy and convenient to measure experimentally.

2.1.3.1.3.2 Solubility Parameters and Interaction Parameters

The solubility parameters of a substance are a measure of the attractive forces between the molecules of the material. The values of solubility parameters can be used to predict affinity between materials. A well-known application is using them to estimate the solvency behavior of a solute in a solvent (Galindo-Rodriguez, Allémann et al. 2004, Brandrup, Immergut et al. 1989). Commonly used solubility parameters include the Hildebrand solubility parameters $\delta$ and the Hansen solubility parameters $(\delta_d, \delta_p, \delta_h)$. Usually, smaller difference in solubility parameters indicates higher affinity of materials and higher mutual solubility and miscibility. In practice, many solubility parameters for common solvents and polymers are readily available in the literature (Barton 1983, Hansen 1971, Wu, Brandrup et al. 1999).

The Hildebrand solubility parameter is defined as an approximation of the geometric mean of the cohesive energy density of the material, which reflects the Van der Waals force needed to hold the molecules together (Burke 1984, Hildebrand, Wood 1933). In Motoyoshi’s work, the Hildebrand solubility parameter $\delta$ of polymers and water (non-solvent) are used to justify the resulting structure of polymer blend particles (Motoyoshi, Tajima et al. 2010). The authors claim that Janus particles form when two composing polymers have similar hydrophobicity, as indicated by close values of $\delta$. If two polymers have large differences of $\delta$, they form particles with core-shell structure, with the shell forming from the one having a value of $\delta$ that is closer to that of water. Analysis shows
certain correlation of the differences of values of $\delta$ with experimental findings, but not completely.

Hansen solubility parameters extend Hildebrand solubility parameters into three parts by considering the composition of forces as follows (Burke 1984, Hansen 2000):

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2$$

Where $\delta_d$, $\delta_p$ and $\delta_h$ each considers the interaction components of dispersion, polar and hydrogen bonding. The difference in Hansen solubility parameters between solvent and non-solvent was investigated to see its correlation with the particle size behavior and was defined as (Galindo-Rodriguez, Allémann et al. 2004):

$$\Delta\delta_{GS-NS} = \left[\left(\delta_{d,GS} - \delta_{d,NS}\right)^2 + \left(\delta_{p,GS} - \delta_{p,NS}\right)^2 + \left(\delta_{h,GS} - \delta_{h,NS}\right)^2\right]^{1/2}$$

GS and NS stand for solvent and non-solvent. In the polymer system in Galindo-Rodriguez’s work, it was shown that the larger value of $\Delta\delta_{GS-NS}$, obtained by varying the solvent types, the larger the nanoparticle size, with a few exceptions and outliers.

The interaction parameter $\chi$ is directly related to the enthalpy of the free energy of mixing (Bates 1991). A simple form of the interaction parameter was used by Galindo-Rodriguez et al. to estimate the affinity between solvent and non-solvent for a polymer system (Galindo-Rodriguez, Allémann et al. 2004, Martin, Bustamante et al. 1993):

$$\chi_{GS-NS} = V_{GS}(\delta_{GS} - \delta_{NS})^2/RT$$

which was calculated from known $\delta$ values of solvent and non-solvent, and the molar volume of solvent $V_{GS}$, when only the solvent was varied while the non-solvent was kept...
the same. Choi et al. used a similar expression for the interaction parameter for polymer and solvent (Choi, Kwon et al. 2002):

\[ \chi_{GS-P} = V_{GS}(\delta_{GS} - \delta_P)^2/RT \]

In this equation, P stands for polymer and \( V_{GS} \) is the molar volume of the solvent since the solvent was varied in the systems investigated.

It is found that they have good correlation with the final particle size behavior in certain polymer systems (Galindo-Rodriguez, Allémann et al. 2004, Choi, Kwon et al. 2002), that is: larger \( \chi_{GS-NS} \) and smaller \( \chi_{GS-P} \) is correlated with larger nanoparticle size. Larger \( \chi_{GS-NS} \) and smaller \( \chi_{GS-P} \) usually indicates less affinity between solvent and non-solvent and higher affinity between solvent and polymer macromolecules, so the diffusion of solvent to non-solvent phase is hampered and more solvent partitions in polymer particles, which leads to large particle size.

For the solubility parameters and interaction parameters mentioned above, good correlation is achieved in the examples. However, this does not justify simply expanding the findings in specific systems to general cases without knowing the characteristics and limitations of these parameters. For instance, it is well known that the Hildebrand parameter is good for non-polar substances while Hansen solubility parameters are more powerful for polar materials (Koenhen, Smolders 1975). Hildebrand solubility parameters neglect the composition of the Van der Waals force and only take into account the total strength of the force, while small values of the enthalpy of mixing requires the similarities of molecular polarity both in the polar strength and the polar composition (Hansen 2000, Burke 1984). In addition, Hildebrand relates the energy of
mixing to the measurable energy of vaporization of the material, which approximates the attractive forces in pure substances only (Koenhen, Smolders 1975). This leads to the neglect of unexpected interactions from combining separate components (especially hydrogen bonding in certain mixtures). The interaction parameters defined above based on the Hildebrand solubility parameters are always positive due to the simplifications are made based on Hildebrand approach, while in reality certain types of interactions among components can result in a negative interaction parameter (Bates 1991). For polymers, reported solubility parameter of the same polymer may vary greatly since it is a complex function of many variables (e.g., polymer molecular weight, degree of crystallization, extent of branching and temperature, etc.) and it can vary with measurement techniques and conditions (Brandrup, Immergut et al. 1989). This also explains the fact that reported values of Hildebrand and Hansen solubility parameters do not always follow their theoretical relationship. Solubility parameters can be difficult and laborious to determine, so it is impractical to measure the variation for every polymer (Koenhen, Smolders 1975).

When studying the effect of solvent or non-solvent within one homologous chemical series, the parameters relating to both dielectric constant and solubility parameter mentioned above show better correlation with particle behavior and sometimes the correlations show similar trends (Bilati, Allémann et al. 2005). This is because chemicals of the same type share similar chemical nature; hence they exhibit similar bonding characteristics and molecular associations with other molecules in the mixture, so that the essence of the change of polarity can be captured by these parameters (Gorman, Hall 1964).
2.1.3.1.3.3 Other Parameters

Other expressions for the interaction parameter or ways of determining its value are available (Lai, Lin et al. 1998, Bates 1991). One, which is based on the Hansen solubility parameters, uses a combination of $\chi_H$ and $\delta_h$ to understand the mixing of a polymer solution. $\chi_H$ is a Flory-Huggins type correction parameter (enthalpy correction) (Koenhen, Smolders 1975, Chen 1971):

$$\chi_H = \frac{V_m}{RT} [(\delta_{d1} - \delta_{d2})^2 + (\delta_{p1} - \delta_{p2})^2]$$

which considers contributions to the energy of mixing caused by dispersion and dipole forces. $\delta_h$ is for hydrogen bonding or donor-acceptor interactions and it is considered separately. For instance, pure electron donor or acceptor types may have a small $\delta_h$ by themselves, but when they are in close contact with each other, a complex can form due to strong hydrogen bonding between them (Koenhen, Smolders 1975). More ways of estimating interaction parameters are mentioned in Lai’s work (Lai, Lin et al. 1998). It is claimed that both $\chi_{NS-GS}$ and $\chi_{GS-P}$ are better treated by considering concentration dependent features, with the value of $\chi_{NS-GS}$ calculated theoretically from the change of free energy and the value of $\chi_{GS-P}$ estimated by vapor-sorption experiment or osmotic pressure experiment. Meanwhile $\chi_{NS-P}$ was determined by equilibrium swelling experiments and it is widely accepted that $\chi_{NS-P}$ can be considered as concentration independent (Lai, Lin et al. 1998). These parameters were used to theoretically construct the phase diagram as shown in Figure 2.2 (a) and a good match was achieved with experimental findings. These interaction parameters determined are considered as more...
accurate and, however, they can be very laborious both theoretically and experimentally and are not always practical to use.

Other parameters that relate to the nature of the materials, such as the diffusion coefficient and viscosity, have also been studied, with other factors such as concentrations, temperature and mixing condition kept the same, since they also affect these parameters. For instance, the exchange ratio \( R \) is found to correlate well with particle size. It is defined as:

\[
R = \frac{D_{GS-NS}}{D_{NS-GS}}
\]

\( D \) is the mutual diffusion efficient (from solvent to non-solvent and reverse). It is calculated from equations relate to viscosity and molar volumes. A larger \( R \) is similar to a smaller \( \chi_{GS-NS} \), which indicates better interdiffusion of solvent and non-solvent and leads to smaller particle size (Choi, Kwon et al. 2002). More studies of the effect of viscosity (intrinsic viscosity) and diffusion coefficient of solvents can be found in work of Thioune et al. and Bech-Broichsitter et al. (Thioune, Fessi et al. 1997, Beck-Broichsitter, Rytting et al. 2010)

2.1.3.1.3.4 Summary

In all, the parameters mentioned here do not perfectly correlate with particle behavior in nanoprecipitation, mainly due to the underlying simplifications of the parameters. The simplifications are unavoidable and necessary for analysis to be practical and workable. Dielectric constants, solubility parameters and other parameters each reflect certain aspects of the interactions. The degree of correlation between the parameters and the
particle behavior depends on how well these parameters capture the information about interactions among the materials used. For some types of systems, simple parameters with readily available or easy to measure values are enough to capture the essence of the interactions; hence good correlation can be achieved. In other systems, more complicated forms of parameters and special attention are needed, which means more work both theoretically and experimentally are required or the understanding is just limited by the availability of certain information.

Finally, although some literature discusses the correlation of absolute dielectric constant or solubility parameter with particle behavior explicitly, it is always necessary to understand their findings in the context (that is, the specific system of discussion) before attempting to generalize the findings to other systems. Usually the parameters capturing the interactions among components serve as more direct indicators than individual parameters for nanoprecipitation behavior.

2.1.3.2 Concentrations and Ratios

The concentrations and mole fractions of the components are widely studied in the literature on nanoprecipitation, mainly in terms of their effect on particle size. The exact relationship between particle size and concentrations in nanoprecipitation depends on various factors, such as the supersaturation level, the material types, the mixing conditions and so on. Under certain conditions, a clear dependence of particle size on concentrations is found.

In the case of high supersaturation and sufficient mixing within the ouzo region, it has been found that particle size increases with the initial solute concentration $f_s^i$ or the final
solute concentration $f_s$ (if solvent fraction $f_{GS}$ is fixed)$^1$, until the system hits the ouzo boundary, where aggregation begins to form macro solid particles or causes rapid coalescence of liquid droplets (Galindo-Rodriguez, Allémann et al. 2004, Aubry, Ganachaud et al. 2009, Van Keuren, Bone et al. 2008, Beck-Broichsitter, Rytting et al. 2010, Vitale, Katz 2003). Similar findings are also found in composite nanoprecipitation systems (Sosa, Liu et al. 2016, Molpeceres, Guzman et al. 1996). However, another essential factor, the “ratio of excess solute to solvent” $^2 \frac{f_{excess\,S}}{f_{GS}}$ is found to better correlate with particle size than $f_s^i$ (Vitale, Katz 2003, Beck-Broichsitter, Rytting et al. 2010):

$$\frac{f_{excess\,S}}{f_{GS}} = (f_s - f_s^*)/f_{GS}$$

where $f_{excess\,S}$ is the concentration of solute in excess of its saturation in the final mixture and $f_s^*$ is the equilibrium solute concentration in the final mixture. Note that in discussion of polymer systems, many reports, such as work of Aubry et al. and Beck-Broichsitters et al., use the initial polymer concentration $f_p^i$ as a good approximation of $\frac{f_{excess\,P}}{f_{GS}}$ and these two ratios are used interchangeably, since the term $f_s^*$ is comparatively small and can be neglected in this case (Aubry, Ganachaud et al. 2009, Beck-Broichsitter, Rytting et al. 2010). This approximation is less appropriate for low supersaturation cases

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$^1$ For the initial molecular solution, the superscript i is used. $f_s^i$ refers to the initial solute concentration in the solvent phase, it equals the ratio of solute to solvent and is usually in unit of mass ratio or mass/volume (mg/ml). For the final solution, $f_s, f_{GS}$ and $f_{NS}$ stand for the solute fraction, solvent fraction and non-solvent fraction in the final mixture, usually in unit of mass fraction or volume fraction. In nanoprecipitation systems, the ratio of solvent to non-solvent ($\frac{f_{GS}}{f_{NS}}$) is considered to convey the same information as solvent fraction and their relation approximates: $\frac{1}{f_{GS}} = 1 + 1/\frac{f_{GS}}{f_{NS}}$. This is valid since the solute fraction is usually very low compared to that of the solvent and non-solvent fractions.

$^2$ The ratio of excess solute to solvent is also known as the “excess solute fraction/GS fraction” (Botet
(esp. when solvent fraction is high) or for cases where $f_s^*$ is not negligible, such as the system of Vitale et al. (Vitale, Katz 2003).

Diluted initial molecular solutions (low $f_s^l$) are usually required in polymer nanoprecipitation to form nanoparticles (Legrand, Lesieur et al. 2007, Galindo-Rodriguez, Allémann et al. 2004). This is because the viscosity of the initial solution increases with polymer concentration and it is believed that a sufficiently low viscosity of the solvent phase is necessary to suppress polymer chain entanglements (Ganachaud, Katz 2005, Bilati, Allémann et al. 2005).

The solvent fraction affects the ouzo boundary (the critical initial solute concentration), particle size behavior, the equilibrium concentration of solute in the final solution $f_s^*$ and the solvent partition in final particle (if the solvent is not removed). For instance, the correlation behavior between solute concentration and particle size may vary under different regions of solvent fraction level, as observed in the PMMA/THF/Water system (Aubry, Ganachaud et al. 2009).

For composite nanoprecipitation systems, the ratio between solute components affects particle morphology as observed for PS-blend-PI nanoparticles of Prud’homme’s group (Sosa, Liu et al. 2016). In addition, the efficiency of drug ingredient entrapment was found to depend on the ratio of the amount of polymer to the amount of low molecular weight drug ingredient (Molpeceres, Guzman et al. 1996).

**Summary**

The concentrations of the components play a critical role in controlling particle size and the ouzo boundary. This is consistent with the understanding that both solute
concentration and solvent fraction affect the interactions among components, as mentioned in previous discussion of interaction parameters. According to Lai et al., interaction parameters $\chi_{NS-GS}$ and $\chi_{GS-P}$ are concentration dependent and for most polymer solution, $\chi_{GS-P}$ increases as the polymer concentration increases (Lai, Lin et al. 1998).

The correlation between concentrations and particle size is system condition dependent. Their correlation is often used as evidence to justify the formation mechanism proposed of a particular system. A more comprehensive discussion will be laid out together with the formation mechanism later in polymer specific and small organics specific sections.

### 2.1.3.3 Mixing

Mixing is an important controllable factor for nanoprecipitation. It can have a large effect on particle size and reproducibility.

Mixing can be categorized as macro-mixing, meso-mixing and micro-mixing, corresponding to the achievable length scale of the flow stream of decimeters, millimeters or micrometers respectively (Bałdyga, Bourne 1999, Horn, Rieger 2001). Figure 2.3(a) is a simulation of micro-mixing by turbulent mixing at early stages (Horn, Rieger 2001, Gerlinger, Schneider et al. 2000). It can be seen that local lamellae and boundary layers exist initially. A better degree of mixing requires higher mixing energy density input. The mixing energy density can be estimated by the specific energy dissipation rate, which can be used to calculate the characteristic length scale of micro-mixing called the “Kolmogorov turbulent eddy size” (Brick, Palmer et al. 2003).
As described in the introduction, a wide variety of setups are available for achieving different mixing conditions in nanoprecipitation. Among them, impinging jet mixing is considered as being able to achieve the highest mixing intensity (micro-mixing) by intense collision of high velocity liquid jets in a small mixing chamber to generate high intensity turbulent mixing (Brick, Palmer et al. 2003, Zhang, Pansare et al. 2012). As shown in Figure 2.3 (b), the apparatus in Brick’s work can deliver a max flow rate of 20g/s with fluid velocity of 200 m/s. A stirred vessel is considered relating to macro-mixing (Baldyga, Bourne 1999). Also in work of Brick et al., mixing in a stirred vessel with syringe injection was compared with impinging jet mixing and it was found that the mixing intensity of the stirred vessel is much smaller under the typical conditions of impeller speed varying from 50 - 400 revolutions/min and moderate feed rates of 0.5 g/s (Brick, Palmer et al. 2003). For conditions of pouring, injecting or dropping one phase into another phase without stirring, macro mixing can sometimes be achieved, while at other times uneven mixing can occur due to accumulation of one phase near the point of injection (Molpeceres, Guzman et al. 1996), as when a very small amount of one phase is added into the other. In case of liquid nanoprecipitation, it was done by rapidly pouring the non-solvent phase into the solvent phase without stirring, since stirring can cause droplet breakup (Vitale, Katz 2003). Controlled slow mixing or slow solvency change is also applied to control particle size and morphology, such as in the cases of microfluidics, dialysis method and self-organized precipitation method (SORP). In these cases, the assumption of uniform mixing is no longer appropriate since the phase separation of solute happens locally at the flow stream boundary or the interface through diffusion. In microfluidics, laminar flow of the streams can be achieved and interdiffusion of solvent
and non-solvent leads to precise particle formation at the flow interfaces. Meanwhile, turbulent and micro-mixing can also be realized in microfluidics with tesla structures at a high injection rate. The exact mixing level of various set ups relates to details like flow rate and geometry.

A common finding is that particle size decreases with higher energy of mixing (Brick, Palmer et al. 2003, Whittemore IV, Jones et al. 2015). In the case of polymer nanoprecipitation using syringe injection in a stirred vessel, increasing the injection rate can reduce the particle size in the range of a few hundred nanometers (Beck-Broichsitter, Rytting et al. 2010). Similar correlation is observed by Molpeceres et al.: the higher the injection rate, the smaller particle size, but if the injection rate increase beyond some limit, the change of particle size is not obvious (Molpeceres, Guzman et al. 1996). For small organics systems using a stirred vessel, Brick et al. changed the stirring rate in a moderate range and found that particle size is relatively insensitive to the stirring rate. The work of both Molpeceres and Brick found that there is a particle size limit that can be achieved in a stirred vessel, for instance in Molpeceres’s system, the particle size was
always larger than 100 nm (Molpeceres, Guzman et al. 1996, Brick, Palmer et al. 2003).

Meanwhile, Brick found that using impinging jets can generate smaller sizes than stirred vessels (particle size of 100 nm in an impinging jet mixer verses size of 200 nm in a stirred vessel) (Brick, Palmer et al. 2003). Poor mixing may lead to mixture of regions of different composition and the phase separations evolving at different rates, which causes a large size distribution in a system (Roger, Botet et al. 2013).

It can be seen that the details of how particle size relates to mixing conditions is system specific and depends on factors like the range of injection rate, the setup geometry, the material system and so on. Currently, the initial mixing details of nanoprecipitation and its effect are still not well understood. The difficulty lies in many aspects: the short mixing timescale in typical setups makes experimental study very difficult; the effect of initial mixing on final particle size might be compensated or augmented by later growth or aggregation stage so that the value of a phenomenological approach is limited; and the complicated nature of nanoprecipitation systems makes theoretical analysis or simulation very challenging. More discussion of the initial mixing will be laid out in combination with the discussion of the formation mechanisms in later sections, since it is of critical importance in understanding the underlying assumptions of the proposed formation mechanisms.

The choice of mixing set up and desired level of mixing depends on various considerations. When the desired particle size is in 10’s of nanometers, high intensity mixing by impinging jets is preferred. If the intended particle size is in the submicron range and energy cost and simplicity of set up are important, stirred vessels or simply
pouring the constituents together can be considered. Sometimes mild mixing is preferred when sensitive and vulnerable ingredients are involved, such as proteins.

2.1.3.4 Additives

Additives have often been used in nanoprecipitation systems. They can affect particle growth, particle stabilization and the efficiency of ingredient encapsulation. Here, the discussion is limited to surfactants (non-ionic) and electrolytes only. Moreover, since additives relate to stabilization mechanisms of the systems, they will be discussed again in the formation mechanism section.

2.1.3.4.1 Surfactants

As stated previously, one advantage of nanoprecipitation is that for many systems, a surfactant is not necessary to achieve a stable nanodispersion. Other material systems may require surfactants to help stabilize the nanoparticles, especially low molar mass compounds or polymers without ionizable groups (Schubert, Delaney Jr et al. 2011, Roger, Eissa et al. 2013). In much of the literature, surfactants are also used as another controllable factor for particle size and stability, especially for systems in which the surfactant does not affect the application needs. There are four types of surfactants, anionic, cationic, zwitterionic and non-ionic. The discussion here focuses on the non-ionic type. Non-ionic surfactants are usually amphiphilic molecules which help stabilize particles by a steric stability mechanism. For instance, one type of non-ionic surfactant has moderate hydrophilic and lipophilic parts. The lipophilic parts are adsorbed onto the hydrophobic particle surface and the hydrophilic parts help stabilize the particles in the

A common finding in many nanoprecipitation systems (both polymers and small organics) is that the concentration of surfactant or the ratio of solute to surfactant does not have an obvious effect on particle size as long as the surfactant concentration is larger than the critical micelle concentration (Beck-Broichsitter, Rytting et al. 2010, Ganachaud, Katz 2005, Aubry, Ganachaud et al. 2009, Molpeceres, Guzman et al. 1996, Fessi, Devissaguet et al. 1992). Aubry et al. found that the ouzo boundary is independent of the surfactant/PMMA ratio (from 0.1 to 14 of weight fraction) (Aubry, Ganachaud et al. 2009).

Details of how exactly surfactants affect the particles are system dependent. There are several possible models illustrated in the review article by Horn and Rieger, depending on the interaction characteristics among the materials, the time scales of mixing and interdiffusion of the molecules (Horn, Rieger 2001) In the literature on nanoprecipitation involving surfactants that are reviewed here, the authors implicitly assume that the surfactant does not complicate particle formation, but only is adsorbed or coated onto the nanoparticles surface later on to stop particle growth and provide stabilization. The assumption is plausible for these cases based on their findings mentioned above: that particle size and ouzo boundary is independent of surfactant concentration. This assumption will be recapped in later discussion of formation mechanism, but it may not always be valid.
2.1.3.4.2 Electrolytes

For electrostatically stabilized nanoprecipitation systems, adding electrolytes will have a large influence on the particle size and stability, since the electrolytes affect the ionic strength and pH of the solution. Solution pH affects the level of ionization of the ionizable surface groups, while the ionic strength affects the electric double layer of the particles; hence both of them affect the electrostatic interactions between particles (Roger, Botet et al. 2013). For instance, Zhang et al. investigated nanoprecipitation of polymers with ionizable groups on the macromolecules and controlled the ionic strength of the solution by adding salts. He found that the particle size increases with the amount of sodium chloride added (Zhang, Pansare et al. 2012). This finding agrees with work of Roger et al. (Roger, Eissa et al. 2013).

For surfactant (non-ionic) stabilized systems, some groups found that changing pH or ionic strength was almost irrelevant to the final particles (Bates 1991, Fessi, Devissaguet et al. 1992). In work of Fessi et al., the presence of electrolytes (e.g. sodium chloride) does not induce coalescence or precipitation of polymer nanoparticles.

Electrolytes can also affect the efficiency of ingredient encapsulation. For example, in a modified nanoprecipitation method, Peltonen et al. improved the entrapment efficiency of the sodium cromoglycate (a hydrophilic drug) into hydrophobic polylactide nanoparticles from 10% to 70% by lowering the pH of the outer media with the addition of hydrogen chlorides (Peltonen, Aitta et al. 2004).
2.1.3.5 Summary

Various factors can have profound effects on particle formation and particle properties in nanoprecipitation as discussed above and more work is needed to achieve a better understanding of the factors and their correlation with the final products. Due to the differences of the real nanoprecipitation systems, the dominant factors of each system in effecting the particle formation behavior and what the effects are may vary. As a result, studying a nanoprecipitation with new materials requires an *ab initio* approach to find the right or ideal experimental conditions, even for materials of similar kind. Previous studies can provide valuable guidance to help narrow the scope of trials though, such as in the choice of solvent/nonsolvent combinations, concentrations and ratios to start with, additives to consider and so on.

In addition to the factors discussed above, there are also other important ones that affect the particle behavior in nanoprecipitation systems, including temperature of the solvent or non-solvent (Kasai, Oikawa et al. 1998, Nishida 2011), the incorporation of solvent removal process, etc.

2.2 Proposed Formation Mechanisms

2.2.1 Polymer Nanoprecipitation

2.2.1.1 Introduction and Scope of Discussion

The formation mechanisms being currently proposed for polymer nanoprecipitation are mainly based on single component polymer nanosystems. This section summarizes and compares the key literature that is widely cited in this area, mainly from active research
groups. In this literature, the main approach taken is a phenomenological one. The correlations between final particle size and concentrations serve as key evidence and the formation mechanisms are derived from these correlations. Time resolved evidence is less common, such as particle size growth data.

Most of the reports reviewed here are from studies of the formation of nanoparticles of medium molecular weight polymers, such as the 14,700 g/mole PMMA used in the work of Aubry et al. and Rogers et al. (Aubry, Ganachaud et al. 2009, Roger, Botet et al. 2013). The good solvents used are typically acetone or THF, while the miscible non-solvent is water or an aqueous solution containing additives. The final particle size information (size and size distribution) reviewed here is mainly from static or dynamic light scattering (DLS) characterization techniques. Measurements were carried out on nanodispersions either with or without solvent removal. The difference can have a minor effect on particle size but does not affect particle size trend, so here they are not differentiated explicitly. In case of comparing sizes with varied solvent fractions, caution is still needed when the final size difference is small, since the particle size can slightly increase with solvent fraction merely due to the swelling effect, that is, more solvent partitions into particles. For example, this effect can cause a size difference of 15% in a PMMA/Acetone/Water system (Aubry, Ganachaud et al. 2009). In the time resolved measurement of particle growth in Roger’s work, ultrafast small angle X-ray scattering technique (SAXS) was used in combination with a stop flow device. To get data points within 5ms to 205 ms after mixing, multiple samples with the same conditions were prepared since the minimum time between measurements was 200ms, which is only feasible in systems with good reproducibility (Roger, Botet et al. 2013). Polydispersity is
defined differently in different literature. For instance, $d_V/d_N$ is used in work of Aubry, where $d_V$ and $d_N$ are volume-average and number-average particle size respectively, and a value of $d_V/d_N$ around 1.5 is still considered as low polydispersity (Aubry, Ganachaud et al. 2009). In Roger’s work using SAXS, the relative width or polydispersity is defined as $\frac{\delta_R}{<R>} = \frac{<R>-<R>}{<R>}$, where $<R>$ is mean radius. According to [Roger-H cited 29], $\frac{\delta_R}{<R>} = 0$ is perfectly monodisperse and $\frac{\delta_R}{<R>} < 0.1$ refers to particles that are sufficiently monodisperse to crystallize at high volume fraction.

As shown in Table 2.1 and Table 2.2, the literature can be classified by experimental conditions, which are based on supersaturation level, mixing condition, within or beyond the ouzo region and stabilization agents. These tables also list corresponding results and the proposed formation mechanisms. The first condition is fast mixing and high supersaturation within the ouzo region. It is intensively reviewed here since it is among the preferred conditions for nanoprecipitation and a good portion of reported systems are prepared under this condition. My system is also carried out under this condition, which will be described later in the experimental section. The second one is fast mixing but low supersaturation within the ouzo region. Other conditions such as slow mixing by dropping the non-solvent phase into the solvent phase are also briefly discussed.
2.2.1.2 Fast Mixing and High Supersaturation within The Ouzo Region

<table>
<thead>
<tr>
<th>Condition</th>
<th>Formation Mechanism</th>
<th>Evidence</th>
<th>Representative Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast Mixing</td>
<td>Nucleation (skipped)-Aggregation</td>
<td>Initial: Descriptive Aggregation: $R_{\text{final}}$ on $f_p^i$, $f_s$ with analytical analysis</td>
<td>(Aubry, Ganachaud et al. 2009, Beck-Broichsitter, Rytting et al. 2010)</td>
</tr>
<tr>
<td>High S Within Ouzo</td>
<td>(Similar as above)</td>
<td>Initial: Descriptive Aggregation: Time resolved $R(t)$ (5ms – 205ms with numerical simulation)</td>
<td>(Roger, Botet et al. 2013, Roger, Eissa et al. 2013, Zhang, Pansare et al. 2012)</td>
</tr>
<tr>
<td></td>
<td>Polymer collapse-Hindered Aggregation-Ionic Repulsion</td>
<td>Descriptive, based on $R_{\text{final}}$ mixing</td>
<td>(Molpeceres, Guzman et al. 1996, Quintanar-Guerrero, Allémann et al. 1997, Bilati, Allémann et al. 2005)</td>
</tr>
</tbody>
</table>

Table 2.1: Representative literature and proposed formation mechanisms of polymer nanoprecipitation in experimental condition of fast mixing and high supersaturation within the ouzo region.

2.2.1.2.1 Criteria

Fast mixing is a broad description of the mixing condition referring to the rapid change of solvation power upon mixing initial solution with non-solvent. The following examples from the cited literature are considered fast mixing; they cover mixing levels from micro-mixing to macro-mixing, with the details being system dependent. Examples are: turbulent mixing in a stop flow device (Roger, Botet et al. 2013) or impinging jet (Zhang, Pansare et al. 2012), one-shot pouring of aqueous phase (larger volume) into organic phase (smaller volume) with moderate stirring (stop right after mixing)\(^3\) (Aubry, Ganachaud et al. 2009), dropping organic phase to water phase with stirring\(^4\) (Aubry, Ganachaud et al. 2009, Stainmesse, Orecchioni et al. 1995), or syringe injection with moderate stirring (Molpeceres, Guzman et al. 1996, Bilati, Allémann et al. 2005, Beck-Broichsitter, Rytting et al. 2010, Jung, Breitenbach et al. 2000, Galindo-Rodriguez, Allémann et al. 2004).

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\(^3\) They are process 1 and 4 in work of Aubry et al.

\(^4\) It is process 3 in work of Aubry et al.
Generally, a high supersaturation level within the ouzo region refers to the experimental condition in which the initial polymer concentration \( f_P^i \) is low while still well beyond the saturation limit and the solvent fraction \( f_{GS} \) is small as well. For instance, in process 1 of Aubry’s work, \( f_P^i \) (PMMA in Acetone) is 0.01 or smaller in mass fraction; \( f_{GS} \) is between 0.05 and 0.4 for Acetone in water or \( f_{GS} \) is below 0.25 for THF in water (Aubry, Ganachaud et al. 2009, Roger, Botet et al. 2013). A rough estimation of the supersaturation level is performed by Roger based on the equation \( S = f_P/f_P^* \) and the PMMA/Acetone/Water ternary phase diagram reported by Aubry\(^5\) (since Roger used the same materials as Aubry). For the condition where \( f_P^i = 0.01 \) and \( f_{GS} = 2/3 \), high \( S \) is achieved since \( f_P^* \) is estimated to be very small due to the flatness of the binodal line, which means that a small addition of water above the binodal line produces a large supersaturation (Roger, Botet et al. 2013).

2.2.1.2.2 Proposed Formation Mechanism

There are two major proposed theories on the formation mechanisms in polymer nanoprecipitation under the conditions described above (Table 2.1). One is the “Polymer Collapse – Aggregation mechanism”, usually known as “Nucleation-Aggregation” or the “Ouzo Effect”. The other is the interfacial turbulence mechanism, also be known as the “Maragoni Effect” of nanoprecipitation.

\(^5\) The supersaturation level (\( S \)) of a system directly relates to differences of chemical potentials. For most conditions, supersaturation can be approximated as (Brick, Palmer et al. 2003, Roger, Botet et al. 2013, Horn, Rieger 2001): \( S \cong f_s/f_s^* \), where \( f_s \) and \( f_s^* \) are the actual solute concentration in the final mixture (supersaturated) and the equilibrium concentration in the mixture respectively. \( f_s \) is known since it equals to the actual solute fraction added in the final solution, \( f_s^* \) can be estimated by the point where tie line meets the binodal line for the solute-poor phase from the phase diagram. Hence, an approximate value of \( S \) can be derived, accurate to at least the correct order of magnitude.
2.2.1.2.2.1 “Polymer Collapse – Aggregation” Mechanism

Initial Stage: Polymer Collapse

In the initial stage, a signature of this mechanism is that it assumes that a large amount of stable nuclei are generated and only traces of macromolecules are left dissolved in the solvent mixture (Roger, Botet et al. 2013, Aubry, Ganachaud et al. 2009, Zhang, Pansare et al. 2012). In the model, the polymer macromolecules are well collapsed initially upon the rapid changing of solvency and resulting high supersaturation level, while the collapsed size $R_{\text{collapse}}$ (or $R_{\text{swelling}}$ if swelling) of the polymer macromolecules with high molecular weight is considered large enough to be stable. Some authors use a classical nucleation description of this claim, that is, that $R_{\text{collapse}}$ surpasses the critical nucleation size $R_{\text{critical}}$ and overcomes the nucleation barrier so that a large amount of stable nuclei form spontaneously upon fast mixing. This claim is supported by a preliminary estimation of $R_{\text{collapsed}}$ based on $(R_{\text{collapse}})^3 \sim \frac{\text{molar mass}}{\text{density}}$. For PMMA with molecular weight 14,700 g/mol, $R_{\text{collapse}}$ is about 1.7nm; and if the swelling effect of the polymer in the solvent mixture is also taken into account, $R_{\text{swelling}}$ is about 2.2 nm (Roger, Botet et al. 2013). This order of magnitude of size is considered larger than $R_{\text{critical}}$ under these conditions.

Later Stage: Aggregation and Stabilization

For later stages of growth, the model assumes the systems can be considered as colloidal dispersions that evolves by aggregation of the initially formed stable nuclei, not by molecular addition. Depending on the details of particle interactions, the aggregation
process varies. For polymer systems without ionizable groups and stabilized by steric surfactants, the proposed aggregation process is a Brownian type of diffusion limited cluster aggregation (DLCA) process based on the attractive Van der Waals force (known as “Brownian Coalescence”). For polymer systems with ionizable groups, the system goes through a hindered DLCA process in which both Van der Waals force and additional ionic repulsion are taken into account (known as “Hindered Coalescence”) (Roger, Botet et al. 2013). The major evidence for these two scenarios is provided as follows:

1. Brownian Coalescence in Surfactant (Steric) Stabilized Systems

Theoretically, Brownian coalescence considers both Brownian diffusion and the attractive Van der Waals potential and assumes that all droplets have spherical shape and that all encounters lead to coalescence (Roger, Botet et al. 2013). In the work of both Aubry et al. (process 1 and either acetone or THF as good solvent) and Beck Broichsitter et al., good correlation between the particle size and the initial polymer concentration is observed experimentally, following the behavior \( \log(R) \propto \frac{1}{3} \cdot \log(f_P) \) (Aubry, Ganachaud et al. 2009, Beck-Broichsitter, Rytting et al. 2010). The correlation \( \log(R) \propto \frac{1}{3} \cdot \log \left( \frac{f_{excess,P}}{f_{GS}} \right) \propto \frac{1}{3} \cdot \log(f_P) \) also holds in this case\(^6\) and the slope 1/3 indicates that volume per particle is proportional to \( f_{excess,P} \). To justify the finding, Aubry applied a simplified DLCA model using a Smoluchowski kinetic model for aggregation (same idea as Brownian Coalescence), assuming the nuclei formed initially

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\(^6\) In this case, \( f_P \) approximates \( \frac{f_{excess,P}}{f_{GS}} \) as mentioned in Section 2.1.3.2, and \( f_P \approx f_P^{1/3} \cdot f_{GS} \propto f_P^{1/3} \) when the solvent fraction is constant, since here the amount of polymer can be neglected compared to the amount of solvents.
frequently encounter each other and aggregate to form larger particles. The simple analysis arrives at a relation of \( d^3 \propto \alpha(f_{GS}) \cdot f_p \cdot t \), where \( d \) is particle mean diameter and the rate coefficient \( \alpha(f_{GS}) \) is only a function of \( f_{GS} \). For a system with fixed \( f_{GS} \) and \( f_p \), \( d^3 \propto t \) holds, resulting in the well-known prediction of the DLCA model of particle growth following a power law of time \( R \propto t^{\frac{1}{3}} \) (Harada, Inada 2009, Roger, Botet et al. 2013). A difference is that here Aubry assumes that the aggregation time is a constant value for the same \( f_{GS} \), since the stop time of aggregation is determined by adsorption of surfactant onto nanoparticles, and the initial time is the start time of mixing. As a result, \( d^3 \propto f_p \propto f_p^\dagger \) holds for fixed \( f_{GS} \), which is consistent with the experimental findings. The final nanoparticles are sterically stabilized by the adsorbed surfactants on the particle surface. Note that the above discussion implicitly assumes that the selected surfactants do not affect the formation mechanism as claimed in Section 2.1.3.4 above.

The particle growth by molecular addition (which known as the growth stage in the classical nucleation theory) is rejected in Aubry’s work because the size behavior does not follow the prediction of the nucleation and growth model. The nucleation and growth mechanism predicts that in the high supersaturation region, the number of stable nuclei increases with the initial polymer concentration. But instead of aggregation of stable nuclei, particles grow only by collecting dissolved macromolecules in the solution, since the concentration of nuclei is not high enough and the frequency of their encounter is low (Dirksen, Ring 1991, Aubry, Ganachaud et al. 2009). The growth process stops when dissolved macromolecules are depleted and the final particles are stabilized by the surfactants, since Ostwald ripening is less likely to occur for high molecular weight polymer system in this region (Aubry, Ganachaud et al. 2009). Hence, the nucleation and
growth model predicts a particle size decrease with increasing \( f_P^i \) since the amount of dissolved polymer macromolecules in the high supersaturation region is almost independent of \( f_P^i \), and this prediction contradicts the experimental findings.

2. Hindered Coalescence in Electrostatic Stabilized Systems

When there are ionizable groups available on polymer chains, surface charges are present which affect the particle formation and stabilization in nanoprecipitation. In this case, no external stabilizing agents are needed. Ionizable groups can be either deliberately introduced onto polymer chains or may come from initiators at the ends of the polymer chains (Roger, Eissa et al. 2013, Beck-Broichsitter, Rytting et al. 2010). In work of Zhang et al., polystyrene has sulfate end groups coming from the initiator and the inherent negative surface charge is confirmed by zeta potential measurements (Zhang, Pansare et al. 2012). In work of Roger et al., each PMMA chain carries one carboxylic acid end group from initiators. The negative charge was confirmed by electrophoretic mobility measurements (Roger, Eissa et al. 2013). Electrolytes such as sodium hydroxide or sodium chloride were added into the water phase to control pH to ensure ionization of the end groups and to adjust the ionic strength (Zhang, Pansare et al. 2012, Roger, Eissa et al. 2013, Roger, Botet et al. 2013).

Hindered coalescence suggests that during the particle growth stage, aggregation begins among the abundant stable nuclei or droplets formed during the initial mixing. Initially the frequency of aggregation is similar among droplets with random sizes as in Brownian coalescence; while at later times, the coalescence between large droplets and small droplets begins to dominate and is more likely to happen than coalescence between large
droplets of similar size due to the stronger ionic repulsion (Roger, Botet et al. 2013). The key evidence is provided in Roger’s work based on time resolved data on size and size distribution extracted from SAXS data from 5ms to 205ms. It was found that the particle size grew logarithmically and the polydispersity decreased with time as follows: 
\[ <R> \propto \ln(t) \] and 
\[ \frac{\delta_R}{<R>} \propto \frac{1}{<R>^2}. \]

The experimental data was in good agreement with the fitting by a kinetic Monte Carlo simulation of hindered coalescence. The simulation used the kernel of the Smoluchowski kinetic equation, including both attractive Van der Waals and size dependent ionic repulsive interactions and assuming monodisperse initial condition and a spherical shape of nanoparticles (Von Smoluchowski 1916, Drake 1972). It calculated the probability of coalescence of droplets and showed that it is much higher between large and small droplets than between two large droplets. Zhang et al. arrived at a similar conclusion about hindered coalescence in polystyrene nanoprecipitation, based on the correlation of particle size and stability with ionic strength (Zhang, Pansare et al. 2012). Experimentally, increasing ionic strength leads to a particle size increase and eventually causes the onset of flocculation. This correlation is well justified by calculating the interaction energy between nanospheres if both electrostatic repulsion and attractive Van der Waals force are taken into account (Israelachvili 2011, Zhang, Pansare et al. 2012). The interaction energy is ionic strength dependent. When the ionic strength is low, the calculated energy barrier is large between large nanoparticles while the barrier is only a few \( kT \) between large and small ones. The larger the energy barrier, the lower the probability of coalescence. When the ionic strength is large, the interaction between large and large nanoparticles becomes attractive. This explains the stability and low polydispersity of nanodispersions at low ionic strength and the occurrence of flocculation
at large ionic strength. A point to emphasize is that both Brownian and hindered coalescence mechanisms assume that the particles always return to a spherical shape after coalescence, otherwise particles would grow by reaction limited cluster aggregation process (RLCA). This assumption was confirmed by TEM imaging of the nanoprecipitated polymer nanoparticles (Aubry, Ganachaud et al. 2009).

The growth behavior observed in the above work does not follow the predictions of Brownian coalescence, Ostwald ripening or the reaction limited cluster aggregation process (RLCA just mentioned). Brownian coalescence predicts that the particle size growth follows a power law in time and that the size distribution has a self-preserving form, that is, \( \frac{\delta_R}{<R>} \) remains constant. Ostwald ripening, described by the Lifshitz-Slyozov-Wagner theory, is also a diffusion-limited process and it shares a similar power law as Brownian Coalescence (Woehl, Park et al. 2013, Bansil 1993). For the RLCA process, the particles are predicted to grow exponentially with time \(< R > \sim \exp (t)\) and become clusters with branched structures, with their polydispersity \( \frac{\delta_R}{<R>} \) also increasing over time (Lin, Lindsay et al. 1990).

2.2.1.2.2.2 Interfacial Turbulence Mechanism

An alternate to the “Polymer collapse – Aggregation” mechanism has been proposed: the “Interfacial Turbulence” mechanism, which explains the particle behavior of polymer systems under the conditions of fast mixing and high supersaturation within the ouzo region. The groups using this method to analyze their data include Fessi, Doelker, Kissel and Molpceres, etc. The literature listed in Table 2.1 is from these groups.
In the model, dynamic mixing causes the flow streams of the organic phase to break into droplets of a certain size depending on the level of mixing. Further interdiffusion of solvent and non-solvent leads to high supersaturation locally at the interfacial region. This assumes that phase separation happens and nanoparticles form at these interfaces, which further disperse into the dispersing medium. When the depletion process of solvent from the droplets is completed, the polymer nanoparticles may be stabilized by surfactants or continue into the aggregation process (Horn, Rieger 2001, Quintanar-Guerrero, Allémann et al. 1997). A schematic presentation of this mechanism is shown in Figure 2.4 for a composite nanoprecipitation system with both polymer and organic compound (Horn, Rieger 2001).

This mechanism is used to justify the findings of polymer size dependence on rate of injection in a system using stirred vessel setup and syringe injection (Molpeceres, Guzman et al. 1996). As shown in Figure 2.5, the injection rate varies while the water phase is kept at a moderate stirring rate (500 rpm) to avoid accumulation of the organic phase. It was found that, at the range of low injection rate (e.g. small gauge size and low injection pressure), a clear decrease

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Figure 2.4 Schematic diagram of interfacial turbulence mechanism. Active compound pseudo-latex nanodisperion formation by nanoprecipitation. Reproduced from (Horn, Rieger 2001) with permission of John Wiley and Sons.
of polymer nanoparticle size was observed with increasing injection rate. Within this range the injection flow in the syringe needle was laminar flow since the calculated Re was less than 2400. When further increasing the injection rate and the injection flow in the needle, there was a transition from laminar flow to turbulent flow, and the particle size drop was very obvious. Further increasing the injection rate within the turbulent flow condition did not cause an obvious particle size change; the size was almost independent of injection rate change and the system was unable to achieve particle sizes below 100 nm. The paper applied a descriptive approach to explain the particle size behavior at different mixing conditions as follows (Figure 2.5). First, the mixing upon organic phase entering into the water phase was considered to be always turbulent, even in case of both the flows in the needle and in the stirred vessel being laminar, due to the flow inside the needle being restricted while the water phase was relatively unrestricted. The flow stream of the organic phase hence broke down to droplets of various sizes. In the low injection rate region or the transition region, the higher injection rate led to smaller droplets. The smaller droplets result in the faster completion of depletion of solvent in the droplets by continuous interdiffusion of solvent and non-solvent. Hence the particle size was smaller. In the high injection rate region, it is harder for the set up to further reduce the sizes of the droplets when it approaches its optimum level of mixing. This explains the lower sensitivity of the final particles on further injection rate change and the limitation of the minimum particle size the system can achieve. This argument also supports the behavior that particle size increases with polymer concentration since more polymer macromolecules are available in the droplets or more formed nanoparticles are available for later aggregation in the system.
In the literature supporting the interfacial turbulence model, some attempts to qualitatively relate the local particle formation to the lowering of interfacial tension between phases was done (Davies, Rideal, Quintanar-Guerrero, Allémann et al. 1997). One piece of evidence that was claimed to support this proposal was that the particle size slightly decreased with increasing temperature of the water phase, since the temperature rise causes a decrease in the interfacial tension. In work of Beck-Broichsitter, counter evidence was provided against the interfacial tension proposal (Beck-Broichsitter, Rytting et al. 2010). It claimed that the interfacial tension changes when changing the amount of surfactant added in the water phase or premixing a small amount of solvent into the water phase, hence the particle size should vary accordingly. But experimentally no obvious particle size change was observed. Nevertheless, this evidence is not strong enough to bring any solid conclusion and there are more considerations needed to fully interpret these findings.
2.2.1.3 Understanding and Challenges

2.2.1.3.1 Initial Mixing and Phase Separation Stage

For the polymer nanoprecipitation in case of fast mixing, high supersaturation and within the ouzo region, the initial stage of mixing and phase separation is least understood compared to the later aggregation and stabilization stages.

One key complication is from the fast mixing process. The essential question is whether the assumption of homogenous mixing at the initial stage is valid in real experiments or instead particle formation happens locally at the interfacial layers of different phases where the local supersaturation is high. Fast mixing is a vague description stating that the completion time is short for the system going from the initial state to the final high supersaturation state. But the idea of fast mixing is not equivalent to and cannot guarantee homogeneous mixing before local particle formation. Common fast mixing (turbulent) conditions, such as meso-mixing in stirred vessel or even micro-mixing by high intensity impinging jets, do not automatically ensure homogeneous mixing (Horn, Rieger 2001). It can be seen from above that different researchers have approached this question differently. The “polymer collapse - aggregation” mechanism assumes homogenous mixing is valid before phase separation happens, while the “interfacial turbulence” theory claims local phase separation at the interfaces between solvent and non-solvent phases and particle formation occurs before homogenous mixing. Each hypothesis may be reasonable since the exact condition will be system dependent and a more detailed analysis is needed case by case. The effect of mixing depends on aspects including the level of mixing (achievable length scale), the relationship between the
mixing time and the time scales of interdiffusion and corresponding phase separation and particle formation. If in a system, the time scales of mixing and interdiffusion between solvents are less than the time scale of particle formation, it is not necessary to consider the details of mixing and homogeneous mixing can be assumed. Otherwise, particle formation happens locally at the interdiffusion boundary layers and the details of mixing need to be considered. There has been some experimental and theoretical work done to investigate the mixing level and estimate these timescales. For instance, Johnson et.al carefully investigated and managed to achieve a good understanding of the mixing details of a particular confined impinging jet mixer (Johnson, Prud'homme 2003a). Some other work shows non-classical time dependence of local supersaturation (Horn, Rieger 2001). However, the level of accuracy of these estimations limits their practical value in real cases of fast mixing, especially when the actual timescales are comparable and often not well understood (Horn, Rieger 2001).

The understanding of the initial polymer collapse and the details of phase separation are also limited (Horn, Rieger 2001). It can be seen from above that in both formation mechanisms listed, only a rough description of the initial polymer particle formation is provided, and very little details or direct evidence are available. Most work does not contain detailed discussion and just assumes that mixing happens very fast and that the collapsed macromolecules are stable (in terms of the classical nucleation theory, it is assumed that the collapsed size of the macromolecules is larger than the critical nucleation size and so the nucleation process is not considered). In the interfacial turbulence mechanism, most work does not mention whether the polymer nanoparticle formation at the interfaces happens by nucleation and growth, spinodal decomposition or
some other mechanism. In work of Aubry et al., it is claimed that the initial phase separation is by nucleation, not spinodal decomposition (Aubry, Ganachaud et al. 2009). The supporting evidence is that the experimentally measured ouzo region is located within the metastable region and the dilution pathway of the system does not cross the theoretically calculated spinodal line, which indicates that the initial phase separation cannot be by spinodal decomposition. However, it is questionable to rule out spinodal decomposition based on the evidence just mentioned even in that particular system of discussion, not to mention other systems. This is because, first, the location of ouzo boundary is determined based on final particle behavior, which is not only affected by the initial phase separation but also by later stage particle growth and particle stabilization. This relates to the drawback of the phenomenological approach that will be mentioned later. Second, the accuracy of the calculated spinodal line depends on level of theoretical simplifications and reliability of values of parameters (Lai, Lin et al. 1998), especially when the system under investigation is not simple.

Above all, the initial stage of nanoprecipitation in this condition is not yet well understood. Current knowledge is mostly derived from final particle behavior dependence on the preparation parameters (a phenomenological approach). The shortcoming of the phenomenological approach is that the final particle behavior carries information on both the initial stage and the later stage, and it can be hard to differentiate them and retrieve the information just for the initial stage. It is possible that different formation paths result in similar end products or that the sensitivity of the end products on initial condition is not profound enough to be detected or that its effect is compensated or augmented by later growth and aggregation stages.
Due to the fast and complicated nature of the initial stage, it is almost impossible to get direct information both experimentally and theoretically. In many systems, the initial process can happen and finish within milliseconds, the mixing is dynamic and produces strong background noise and the initial particle size is very small; the combination of all these is beyond the capability of current available measurement techniques such as DLS or SAXS. Even though the measurement of SAXS can go down to 5 ms after mixing, the first time resolved particle size measured is already much larger than estimated size of a collapsed single macromolecule. Theoretically, existing theories based on thermodynamics fail to quantitatively match the experimental findings. As stated above in section 2.1.2.3, the nanoprecipitation is a nonreversible off-equilibrium process, and the kinetics cannot be neglected (Botet 2012). Ideally, numerical work at the molecular level can be used to study the initial process with a certain level of simplification. Computational studies of the evolution of nanoprecipitating systems can be done for short times for a small system (Botet 2012). However for the initial stage of polymer nanoprecipitation in fast mixing condition, it is practically very challenging to simulate. At the initial stage, the polymer macromolecules cannot be treated as hard spheres yet and the full conformations of the polymers must be taken into account. More difficulties come from the complex nature of dynamic mixing and the necessity to consider numerous factors and their interactions in a large complicated system. This requires large computational resources and a good understanding of the interactions.

2.2.1.3.2 Later Particle Aggregation and Stabilization Stage

The growth stage of polymer nanoparticles is better understood than the initial stage. Experiment techniques are capable of directly measuring the time resolved particle
behavior in this stage since the system evolves at a much slower pace. Meanwhile, it is much easier to apply numerical methods, since the system’s behavior can be well described by coarse-grained models and polymer nanoparticles can be viewed as colloids without the need to consider details at the atomic level (Jones 2002, Roger, Botet et al. 2013). In recent findings as discussed above, the theoretical work on aggregation process explains the experimental particle behavior well, especially in the electrostatic stabilized systems (Roger, Botet et al. 2013, Zhang, Pansare et al. 2012). Depending on the specific systems, the aggregation process may or may not occur before particles become stabilized. Note that although the aggregation is not discussed in detail in the interfacial turbulence mechanism, the findings of polymer aggregation can also be applied as a following step before the particles are stabilized. However, caution is needed to accept the findings in surfactant stabilized system, since it might be an oversimplification to assume that the stabilizing agents only help stabilize the polymer nanoparticles by adsorbing onto the particle surfaces and do not complicate the formation mechanism.

Thermodynamically, phase separation continues as the system minimizes interfacial free energy and relaxes to macroscopic phase separation at equilibrium. This leads to the formation of large aggregates or complete phase separation. Hence, to achieve metastable dispersions of nanoparticles, it is required to have interactions that can restrict phase separation on the colloidal scale (Roger, Botet et al. 2013). In most work, the metastability is realized either by having ionizable groups on the polymer macromolecules or using stabilizing agents. The final nanodispersions can be stable for days or months without noticeable particle size change or aggregation. If the polymer
macromolecules cannot provide any charge and not stabilizing agents are introduced, the system shows no metastability (Roger, Eissa et al. 2013, Roger, Botet et al. 2013).

2.2.1.4 Other Conditions and Variations of Polymer Nanoprecipitation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Formation Mechanism</th>
<th>Evidence</th>
<th>Representative Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast Mixing Low S (Within Ouzo)</td>
<td>Nucleation-Growth-Surfactant adsorption</td>
<td>Descriptive, based on $R_{\text{final}}$ on concentrations</td>
<td>(Aubry, Ganachaud et al. 2009)</td>
</tr>
</tbody>
</table>

Table 2.2 Representative literature and proposed formation mechanisms of polymer nanoprecipitation in other experimental conditions

2.2.1.4.1 Fast Mixing and Low Supersaturation within The Ouzo Region

The criteria for fast mixing within the ouzo region are the same as discussed previously. Low supersaturation within the ouzo region is usually realized by using a larger solvent fraction and a low polymer concentration. For instance, when $f_{GS}$ is larger than 0.25 in the PMMA/THF/Water surfactant stabilized system in the work of Aubry, the system behaves differently than it does in the high supersaturation region (Aubry, Ganachaud et al. 2009). Note that the low supersaturation condition is very narrow and may not always occur within the ouzo region. For example, when acetone is used as good solvent instead of THF in the same PMMA/Water system, the entire ouzo region is considered to be within the high supersaturation region according to the particle size behavior. This may relate to the difference of solubility of PMMA in the acetone water mixture and the THF water mixture.

Aubry et al. found that nanoparticles form in this region, and that the particle size is independent of the initial polymer concentration and increases with solvent fraction. This
observation does not follow the prediction of the aggregation process. The nucleation and
growth formation mechanism was proposed by the author to rationalize the particle
behavior. Since more solvent is present in the mixture, the critical nucleation size
becomes larger and the PMMA macromolecules become more flexible and more of them
are dissolved. As a result, it is less accurate to assume that polymer macromolecules are
well collapsed as stable nuclei initially upon mixing, this is different from the case in the
high supersaturation region. Since the events of formation of stable nuclei decrease, so do
the frequency of encounter or aggregation among the nuclei that are further apart. It is
more likely that the particles grow by collecting isolated single macromolecules and
maybe in combination with a certain level of nuclei aggregation. The independence of
particle size on initial polymer concentration can be explained with the nucleation and
growth model by considering two compensating effects: a higher initial polymer
concentration leads to higher supersaturation and hence more nuclei, while more polymer
macromolecules are available for growth. The particle size increases with THF fraction
can also be rationalized as being due to the critical nucleation size increasing and the
number of stable nuclei decreasing, with more dissolved macromolecules available for
particle growth.

This mechanism is plausible in explaining the findings of Aubry, while more direct
evidence is needed to fully understand the process in this region, such as time resolved
particle growth data. Still, it might be system dependent and other formation mechanisms
might take place in other systems under the case of fast mixing, low superaturation and
within the ouzo region.
2.2.1.4.2 Slow Changing of Solvation Power

Polymer nanoprecipitation is also often carried out by a slow change of solvation power. This is a preferred way of growing colloidal nanoparticles before the development of fast nanoprecipitation and is utilized to control particle growth and monodispersity by avoiding aggregation (Roger, Botet et al. 2013, LaMer, Dinegar 1950). Slowly dropping non-solvent into the solvent phase is one typical way of achieving this condition (Aubry, Ganachaud et al. 2009). It is believed that the system slowly crosses the binodal line into the metastable region during the slow rise of the saturation level. Near equilibrium, phase separation takes place at a low supersaturation level initially by a nucleation and growth mechanism since the system is within the metastable region. Other cases of this condition include solvent exchange by dialysis, microfluidics with laminar flows or the newly developed self-organized precipitation method (Yabu 2013, Schubert, Delaney Jr et al. 2011, Valencia, Basto et al. 2010, Botet 2012). In these cases, it is believed that interdiffusion of solvent and non-solvent occurs at the macroscopic boundary regions or interfaces of the phases or flow. The supersaturation level gradually changes and controlled phase separation happens at these metastable regions by nucleation and growth. More experimental and theoretical effort is being undertaken to better understand this process, which is less complicated than the fast mixing condition due to the much slower dynamics and non-turbulent nature of mixing (Botet 2012). In practice, the nucleation process is usually heterogeneous nucleation due to presence of foreign nucleation sites.
2.2.1.4.3 Beyond The Ouzo Region

When the initial polymer concentration goes beyond the critical concentration at a certain solvent ratio, macro particles form and coexist with the nanoparticles in the resulting dispersion. One possible explanation is that due to the high polymer concentration, the viscosity of the initial solution (solvent phase) becomes larger and decreases the interdiffusion of solvent and non-solvent. Thus it is harder to achieve homogenous mixing and local phase separation can result in more polydisperse droplets. In addition, the assumptions used to analyze the diluted case may not be applicable to concentrated case, in which the correlations of particles are stronger (Nishida 2011). For instance, the more frequent encounter of particles can result in increased aggregation and less time for polymer particles to return to spherical shape, then the RLCA is more likely to take place and form branched structured flocs. Increasing the number of polymer chains may affect polymer-polymer inter or intra interactions, especially if overlapping of polymer chains occurs (Galindo-Rodriguez, Allémann et al. 2004). Some literature claims that the formation of macro-particles is due to the spinodal decomposition. This claim is rejected by Aubry et al., as the ouzo boundary is found necessarily overlapping with the spinodal line, which indicates a different nature of formation.

2.2.1.5 Summary

While much work has been done in polymer nanoprecipitation, most of it has been application oriented or using phenomenological approaches to study the fundamentals. It is challenging to reconcile the findings reported and get a clear understanding of the formation mechanisms since the final particle behavior can be profoundly affected by the
experimental conditions and material systems. Moreover, the complex nature of the process itself and the lack of proper means to study it prevents direct investigation of the process both experimentally and theoretically, especially the initial stage. Many key questions still remain unsolved to date. Nonetheless, current findings reviewed above are of critical value in guiding the experimental design and the development of analytical models, especially for similar kinds of systems.

2.2.2 Small Organics Nanoprecipitation

2.2.2.1 Scope of Discussion

The study of small organics, including various kinds of dyes, provitamins and active drug ingredients, is also a very active area of nanoprecipitation (Horn, Rieger 2001, Van Keuren, Bone et al. 2008, Brick, Palmer et al. 2003). Current efforts share many similarities with the study of polymer nanoprecipitation, including the basics of proposed formation mechanisms and the experimental approaches undertaken. However, many differences also exist due to the differences in the nature of the materials, such as the diffusion timescales, the ease of forming crystal structures, etc. The discussion here mainly focuses on the condition of fast mixing and high supersaturation within the ouzo region (later will be referred as “condition 1”). Other conditions, such as low supersaturation or slow mixing, are more similar to the well-studied crystallization process that can be found elsewhere. The definitions of fast mixing, high supersaturation and the ouzo region are the same as discussed in the polymer section above. The nanoprecipitation processes discussed in this section are mostly carried out by injection or pouring into a stirred vessel or mixing in a device such as an impinging jet mixer. The
material systems reviewed here are mainly organic nanocrystals or dyes from work of Van Keuren’s group and work of Brick et al. (Van Keuren, Bone et al. 2008, Van Keuren, Nishida 2010, Nishida 2011, Brick, Palmer et al. 2003).

2.2.2.2 Proposed Formation Mechanisms

The proposed formation mechanisms are mostly phenomenological as in polymer nanoprecipitation for the fast mixing and high supersaturation condition. Most of the models of formation applied in the literature are classified by the following considerations: i) if homogenous mixing or interfacial turbulence is assumed, ii) whether the initial phase separation is by nucleation and growth or spinodal decomposition, and iii) if the stabilization of the final nanodispersion is done by surfactants or electrical repulsion.

2.2.2.2.1 Nucleation and Growth

For the initial stage of particle formation, nucleation theory is widely applied in understanding the findings of small organic nanoprecipitation under condition 1. Homogenous nucleation, which is based on classical nucleation theory, is often applied with the assumption that the mixing on the molecular level happens rapidly compared to the rate of nucleation (Van Keuren, Nishida 2010, Mori, Miyashita et al. 2009, Brick, Palmer et al. 2003). The system is considered as being brought into the metastable region and local fluctuations of solute concentration lead to spontaneous nuclei formation (Van Keuren, Nishida 2010, Mullin 2001). Only the nuclei above the critical nucleation size are stable in the solution and able to grow, the ones below it are unstable due to the surface energy cost and will dissolve back to solution. Stable nuclei mostly form initially. This is because the nucleation rate depends strongly on the level of supersaturation and
nanoprecipitation is usually carried out in closed systems, these two result in a feedback mechanism in which the nucleation rate decreases exponentially over time as the level of supersaturation decreases. This is also the key to the narrow size distribution in nanoprecipitation according to LaMer and Dinegar (LaMer, Dinegar 1950). The stable nuclei that are formed are evenly dispersed in the solution due to the local depletion effect (Vitale, Katz 2003). Subsequent particle growth occurs by molecular addition (attachment of individual molecules or Ostwald ripening) or coalescence (Van Keuren, Bone et al. 2008). The nucleation and growth mechanism is often used to explain the correlation between particle size and initial concentration. A typical experimental finding in condition 1 is that particle size decreases with the increase of initial solute concentration (Mori, Miyashita et al. 2009, Nishida 2011). This correlation is consistent with the prediction of the nucleation and growth model in the high supersaturation region: the supersaturation level increases with increasing solute concentration, with the additional solute mainly forming more stable nuclei, hence the final particle size becomes smaller. Here, it assumes that the stable nuclei are dilute enough to keep aggregation rate low enough to be negligible within the time after nanoprecipitation while before size measurement. The classical nucleation and growth theory is a simplified model and matches with some systems. It has critical value in understanding the process qualitatively but its capability is limited.

Generally, a more complex mechanism than the simple models is likely (Park, Privman et al. 2001). Most experimental work suggests the importance of heterogeneous nucleation due to the presence of impurities or imperfections on the container surfaces in systems of small organics nanoprecipitation (Keuren, Georgieva et al. 2003). In addition, an
important question is whether pre-nucleation clusters exist during the initial nucleation stage, such as dimers or N-mers below the critical nucleation size that are unstable thermodynamically but can exist for some duration of time. Van Keuren et al. extensively studied nanoprecipitation of Magnesium phthalocyanine (MgPC) and the experimental findings supported the idea that both stable nuclei and unstable pre-clusters form at the initial nucleation stage, with the pre-clusters serving as the source of the growth of the initially formed stable nuclei later (Van Keuren, Bone et al. 2008). These studies utilized the change of optical properties (new absorption bands and strong fluorescence quenching) of MgPC from single molecule to dimers, N-mers and complexes of MgPC with water. Optical measurements (especially the fluorescence quenching, which can be detected as early as 10s of seconds) suggest that molecular association happens at a much faster rate than particle size growth, which indicates the formation of pre-clusters. Existence of pre-nucleation clusters also has been reported elsewhere, both experimentally and theoretically (Myerson, Lo 1990, Erdemir, Chattopadhyay et al. 2007, Horn, Rieger 2001, Izmailov, Myerson et al. 1999).

The later particle growth stage can also be more complicated in certain systems than described in classical nucleation theory. In the work of Nishida et al. on tetracyanoquinodimethane (TCNQ) nanoprecipitation, it was found that the growth of TCNQ may follow Ostwald’s rule of stages in crystallization (Nishida 2011). This rule states that the initial phase formed by nucleation may not necessarily be the thermodynamically most stable phase (Horn, Rieger 2001). Nishida found that the SEM images of freeze-dried TCNQ (prepared from 40 minute-old dispersion after nanoprecipitation) contain not only the thermodynamically stable polyhedral structure but
also other forms that are very likely the metastable forms resembling the intermediate states of the growth stage, as seen in Figure 2.6 (Nishida 2011). The polyhedral form is the confirmed to be the stable state and the only form found by crystallization directly from acetone solution. The metastable forms evolve over time and proceed to the stable polyhedral state, but the process can be extremely slow, and so these forms can be observed even 40min after nanoprecipitation. The existence of the morphologies other than the polyhedral in nanoprecipitated TCNQ sample also proves the importance of kinetics in small organics nanoprecipitation. It should be pointed out that the existence of metastable phases (Ostwald stages) is only circumstantial and may be system dependent (Ostwald 1897, Threlfall 2003, Nishida 2011).

![Figure 2.6 SEM images of TCNQ structures resemble the progression of growth. (a)-(f) from seed-like nanostructures to branched nanostructures to final polyhedral microcrystal structure; only selected images are present here, more images can be found in work of Nishida. Reprinted from (Nishida 2011) with permission.](image-url)
2.2.2.2 Spinodal Decomposition and Interfacial Turbulence

In work of Brick et al. with dye nanoprecipitation, a different mechanism is proposed for condition 1 (Brick, Palmer et al. 2003). The authors proposed that the system undergoes the interfacial turbulence process initially in combination with a spinodal decomposition phase separation mechanism locally. Upon mixing, the injection stream of initial molecular solution breaks down to small droplets. The size of the droplets depends on the mixing intensity and level of mixing as discussed in section 2.1.3.3. Further thinning out the initial solution streams or droplets to the molecular level is done by interdiffusion of solvent and non-solvent. This is the same as the interfacial turbulence mechanism mentioned in the section on polymer nanoprecipitation, in which the phase separation takes place at the boundaries of the streams and droplets before the completion of interdiffusion and vanishing of the streams and droplets, where the local supersaturation is high. Here it is assumed that the time scale of the solute phase separation is comparatively smaller or close to the time scale of interdiffusion of the solvents. In other words, homogenous mixing before phase separation cannot be assumed. Continue mixing such as stirring helps dispersing the formed nanoparticles into the non-solvent phase.

This interfacial turbulence model is supported by experimental determination of the particle size dependence on the mixing of the system. Different mixing conditions are achieved either by varying the stirring rate (from 50 to 400 revolution per min) in a stirred vessel or by using a high intensity impinging jet mixer. It was found that particle size does not obviously change with stirring rate, while much higher intensity mixing by impinging jets brings the particle size down to around 100 nm from 200 nm in the stirred vessel. This observation contradicts with the idea of homogenous mixing in stirred
vessels, since if homogenous mixing can be assumed; the change of mixing intensity from stirred vessel to impinging jet will not affect the particle size.

At the interfaces of the droplets, Brick claimed that spinodal decomposition is responsible for local phase separation and nanoparticle formation. Unlike nucleation and growth, spinodal decomposition is a process of spontaneous phase separation without a nucleation barrier. Concentration fluctuations grow exponentially and grow most rapidly at an optimum fluctuation length scale, known as critical fluctuation length (CFL), as a result of a balance of interfacial energy cost and mass transportation time (Jones 2002). Brick proposes that the spinodal decomposition is able to further explain why the particle size is almost independent of stirring rate in a stirred vessel, while the size can be further reduced by high intensity impinging jet. To confirm the reasoning, the estimated droplet size of the dispersed initial solution is compared with the estimated CFL that determines the average particle size. First, the dispersed droplet size is estimated by the characteristic mixing scale “Kolmogorov turbulent eddy size” (Tatterson 1991, Brick, Palmer et al. 2003). The calculated mixing length scale in the stirred vessel approximates 30 to 50 µm while it is around 0.2 µm in the impinging jet mixer. Next, the typical CFL in the system is estimated to be around 1 µm. Since the estimated CFL is smaller than the droplet size of the initial dispersed solution, each droplet forms a collection of nanoparticles. The sizes of these nanoparticles only depend on the details of the rate of interdiffusion of solvents and the rate of solute concentration fluctuation, not the mixing rate. Hence, the particle size is almost independent of the stirring rate. On the other hand, the droplet size is smaller than the CFL in the impinging jet mixer. In this case, the particle size is determined by the droplet size achieved by intensive mixing rather than the CFL, this
leads to a further size reduction compared to the case of the stirred vessel. Note that the estimation of the value of the CFL (1 µm in this case) is based on the typical value of the final particle size and the assumptions that the solute within one CFL forms one particle and that the particle is stabilized right away by surfactants. This is a very rough estimation but the simplifications are necessary due to the complexity of the spinodal decomposition in interfacial turbulence and the time dependent features of the CFL as a result of diffusivity and concentration change over time (Brick, Palmer et al. 2003). In other words, the interfacial turbulence process happens simultaneously with the spinodal decomposition phase separation process, the dynamic nature of local solvent quality makes the case more complicated than the current understanding of spinodal decomposition. The approximate estimation of the CFL is considered to be valid to an order of magnitude.

2.2.2.2.3 Stabilization

To stabilize the nanocrystals or nanoparticles, surfactants can be used to minimize the effect of later Ostwald ripening or aggregation if they do not interfere with the application needs. In fact, surfactants are often used in small organics nanoprecipitation (Horn, Rieger 2001). For instance, surfactants are used in Brick’s system and the particle growth is found to be not significant over the course of 5 seconds to 1 minute after nanoprecipitation. The particles are stable for days after quenching into additional water to further prevent ripening (Brick, Palmer et al. 2003). Sometimes, stabilization cannot be achieved without using stabilizing agents. For instance, microcrystalline TCNQ structures rapidly appear after mixing and the dispersion is very polydispersed in all trials.
of TCNQ/Acetone/Water nanoprecipitation, which results in the failure of DLS measurement to give reliable size data of TCNQ particles (Nishida 2011).

In some systems, surfactants are not necessary to achieve stability in nanodispersions. For instance, nanocrystal dispersions can be stable for hours or days in the nanoprecipitation of perylene, naphthalene or MgPC (Van Keuren, Bone et al. 2008, Keuren, Georgieva et al. 2003, Keuren, Georgieva et al. 2001). But unlike the case in systems using surfactants, the particle growth can continue for around 30 minutes before the dispersion becomes stable (Keuren, Georgieva et al. 2001, Drain, Varotto et al. 2009). The exact stabilization mechanism of these systems is not explicitly discussed in the original literature; it could be electrostatic repulsion.

2.2.2.3 Summary

The previous discussion in this section is limited to fast mixing and a high supersaturation condition within the ouzo region for nanoprecipitation of small organics. Similar to polymer nanoprecipitation, the understanding of the initial stage under these conditions is not sufficient and most studies have been phenomenological. The above-mentioned mechanisms may be interchangeable and are not necessarily the only ones suitable for explaining the findings of their systems. For example, although Brick argues against the local nucleation and growth mechanism in the system they studied based on evidence that particle size is nearly independent of initial concentration, this exact particle size behavior can be explained by a nucleation and growth mechanism if a balance is achieved between the extra number of stable nuclei formed and the extra amount of solute for growth. Conversely, the findings explained by nucleation and
growth may also be rationalized by a spinodal decomposition description under certain conditions. In addition, the validity of assuming homogenous mixing is system dependent, since it relates to the timescales of mixing, interdiffusion and phase separation. Even though the argument of interfacial turbulence in Brick is quite strong, caution is needed to generalize the findings, since the timescales can be different in other systems. Regardless of whether the conditions can be modeled by homogeneous mixing or interfacial turbulence, the details of the phase separation itself can be either nucleation and growth or spinodal decomposition or a combination of the two (Brick, Palmer et al. 2003). In all, the proposed models are not absolute and are still being developed, and the formation mechanism for a specific system can be a combination of the theories described above. Certain stages may be skipped in some systems, such as skipping the growth stage and directly entering the stabilization stage. The difficulties in developing a simple model are mainly due to the complicated and dynamic nature of the process and the lack of suitable experimental methods to probe the details at extremely small time and length scales (Van Keuren, Bone et al. 2008). The initial phase separation can happen within milliseconds based on UV-visible absorption and fluorescent measurements (Mori, Miyashita et al. 2009, Van Keuren, Bone et al. 2008). Similar time scales for the initial phase separation have been found based on estimation of diffusional mixing of small molecules in aqueous solution with a typical diffusion constant $10^{-5} cm^2/s$. It is estimated that it takes less than 1ms to diffuse a distance of 1 μm (the CFL estimated) (Brick, Palmer et al. 2003).

Other questions remain unsolved. One relates to the final particle structure. Sometimes the final particles are nanocrystals, as in systems of perylene or naphthalene (Keuren,
Georgieva et al. 2001, Keuren, Georgieva et al. 2003); while other times the final particles are amorphous, such as for yellow cyanophenyl furanone dye (Brick, Palmer et al. 2003). In more complicated cases such as TCNQ nanoprecipitation, crystals with multiple final structures other than the stable polyhedral structure are produced, such as nanocoils and parallelepipeds, as shown in Figure 2.7(Nishida 2011). Structures other than polyhedral have not been observed in crystallization directly from acetone solution, their appearance may relate to the kinetics of nanoprecipitation. Amorphous particle structures should also relate to the kinetic nature of nanoprecipitation since the crystalline state is thermodynamically favorable. The details of the relationship of kinetics and final particle morphology are not yet well understood. Can Ostwald’s rule be used to explain the formation of amorphous nanoparticles? Or could the amorphous structure serve as an evidence of spinodal decomposition rather than nucleation as claimed by Brick? Moreover, the details of growth by molecular addition and Ostwald ripening are still not fully clear (Nishida 2011). The role of surfactants might be more complicated in small organics system than in high molecular weight polymer systems, since it is possible that the surfactant affects the initial system behavior (Horn, Rieger 2001).

Figure 2.7 Various shapes of TCNQ crystals by nanoprecipitation. (a) nanocoils, (b) parallelepipeds, (c) polyhedral. Reprinted from (Nishida 2011) with permission.
3 COMPOSITE NANOPRECIPITATION SYSTEMS

Two particular systems of composite nanoprecipitation are reviewed here. One is the system of a blend of immiscible polymers and the other is a molecular co-crystal. Fundamental studies on composite nanoprecipitation are scarce, as the systems are more complicated and challenging to study than single component nanoprecipitation systems. Here I present some basics and important findings related to these two co-nanoprecipitation systems.

3.1 Nanoprecipitation of Immiscible Polymer Blends

3.1.1 Overview of Polymer Blend Systems

Polymers are giant molecules which can be found everywhere in our daily life, from natural polymers such as DNA and cotton, to synthetic polymers used as plastics, elastomers and adhesives. A polymer blend is a physical mixture of polymers whose property is usually a combination of the properties of the individual components. Based on the miscibility between the composing polymers, the polymer blends are classified as ‘immiscible polymer blends’ and ‘miscible polymer blends’.

Similar to the solubility, the miscibility of the polymers depends on the free energy of mixing (CARRAHER 2010). Most polymers are found to be immiscible with each other, mainly because most polymers are already highly disordered and the entropy would not change enough to compensate the heat of mixing term (Sperling 2001, CARRAHER 2010). Their immiscibility can be better explained with the Flory-Huggins theory of polymer system (Bates 1991, Jones 2002). As a result, immiscible polymer blends only
appear to be mixed at the macro-level, while at the micro-level the phases of the components separate. For miscible polymer blends, polymers are miscible at the micro-level due to the existence of certain functional groups and the interaction among them, which reduces the enthalpy of mixing. Polystyrene and poly (phenylene oxide) are one example of a miscible blend, since both of them have aromatic rings that tend to stack up (CARRAHER 2010). In addition, polymer miscibility depends on temperature and composition. By changing composition or temperature, some polymers can go from miscible to immiscible and back to miscible.

Polymer blends have been widely applied in industry for various purposes over a very long period. Polymer blends with combine properties provide an economically feasible way to expand the applications of materials. A large variety of polymer blends have been studied, ranging from elastomeric blends to natural product blends, covering the application areas of automotive coating, bumpers, packaging films and so on (Robeson 2007, Sperling 2001). Driven by these applications, different preparation methods of polymer blends have been developed to meet specific needs. A traditional treatment is a mechanical blending technique called ‘melt extrusion’ to produce bulk polymer blends (Utracki 2003). Constituent polymers melts are intensively mixed to achieve blending at micro-scale level. For miscible blends, the polymers mix well into one phase. For immiscible blends, the constituent polymers phase separate into local regions, and depending on their concentration and viscosity, one polymer may form a continuous matrix in which another polymer disperses. To improve the properties, special techniques are developed to stabilize the phase domains from coalescence and to reduce the domain size. For example, in situ formation of a compatibilizer (e.g. block copolymers, graft
copolymers and cross-linked copolymers) called ‘reactive extrusion’ is applied during the extrusion process for stabilizing the blend system by increasing the interfacial adhesion and lowering the interfacial tension between the phases. Other blending methods to produce small blend particles include ‘solvent blending/casting’, ‘latex blending’, ‘blending using supercritical fluids’ and ‘freeze drying’ (CARRAHER 2010, Boudenne, Ibos et al. 2011). ‘Solvent blending’ is carried out by dissolving the parent polymer in a common solvent and the polymer blends are formed upon solvent devolatilization. The technique is great for film formation but is limited by the evaporation rate and rigorous conditions. In the ‘supercritical fluid’ technique, supercritical fluids like carbon dioxide are used to reduce the viscosity of the highly viscous polymer in order to get better dispersion during the blending process. It has been reported that polystyrene-blend-poly (methyl methacrylate) can be formed using this technique (CARRAHER 2010, Boudenne, Ibos et al. 2011). In the ‘freeze drying’ method, the constituent polymers are dissolved in a common solvent, then the solution goes through a quick freezing process in which the polymer mixture has no time to separate. Sublimation is followed to remove the solvents and a blended polymer is left in the end (CARRAHER 2010). The concept of ‘freeze drying’ is similar to the ‘nanoprecipitation’ in some sense, that is, using a sudden condition change to freeze-frame whatever forms in the polymer solution. In case of ‘freeze drying’, the condition change is the temperature; while in case of ‘nanoprecipitation’, it is the solubility.

The idea of co-nanoprecipitation has long existed in achieving polymer related drug-loading nanoparticles (known as pseudolatex nanoparticles), in which the polymer serves as the drug carrier and captures the pharmaceutical ingredient during the
nanoprecipitation (Horn, Rieger 2001). It first appears in the patent of Fessi (Fessi, Devissaguet et al. 1992). Polymer blend nanoprecipitations is relatively new. Most work is still driven by drug delivery applications to include different kinds of polymers into the same nanoparticle. For instance, the nanoprecipitation method is used to form the methoxy poly (ethylene glycol)-b-poly (D,L-lactide) (MPEG-b-PDLL) /methoxy poly(ethylene glycol)-b-poly(E-caprolactone) (MPEG-b-PCL) blends in order to control the drug release efficacy by varying the ratio of the composition polymers. The resulting blend nanoparticles are surfactant free, which is of significance in application for drug delivery (Baimark, Srisuwan 2012). Polymeric Janus nanoparticles with two discernible hemispheres of different polymers have also been demonstrated by nanoprecipitating different forms of PLGA polymers into one nanoparticle using a fluidic nanoprecipitation system (Xie, She et al. 2012).

3.1.2 Nanoprecipitation of Immiscible Polymer Blends

In recent years, some groups have started to look into nanoprecipitation of immiscible polymer blends, and interesting findings are revealed in work from Yabu and Prud’homme groups (Yabu 2013, Sosa, Liu et al. 2016). Note that the homopolymers discussed here have molecular weights above 10,000 g/mol or even larger.

3.1.2.1 Flash Nanoprecipitation

As presented in Figure 3.1, Prud’homme’s group carried out co-nanoprecipitaion of polystyrene (PS) and polyisoprene (PI) homopolymers using an impinging jet mixer under conditions of fast mixing, high supersaturation and within the ouzo region (Sosa, Liu et al. 2016). They found that both Janus and multi-faced PS/PI blend nanoparticles
can form in THF/Water nanoprecipitation (without using surfactant). Size and structure of blend nanoparticles were found to depend on factors such as polymer molecular weight, initial feed concentration and PS/PI ratio. Consistent with the findings from single component polymer systems, blend nanoparticle size increased with initial feed concentration. For particle structure, Janus particles are more likely to change to multi-faced particles at higher initial feed concentration or with polymers of larger molecular weights when the PS/PI ratio was larger. A hypothesis was proposed to explain these findings that follows the interfacial turbulence and spinodal decomposition mechanism. It was proposed that the correlation of particle morphology with the factors is a result of the competition between the time scale of polymer-polymer phase separation by spinodal decomposition and the timescale of vitrification of PS (which relates to the timescale of solvent exchange) within the droplets. The Janus structure is the equilibrium structure as the system minimizes its total interfacial energy. This is because the interfacial energies ($\gamma$) of PS-PI, PS-solvent mixture (THF and water, in this case mostly water) and PI-solvent mixture follow the relation $\gamma_{PS-PI} < \gamma_{PS-mixture} \approx \gamma_{PI-mixture}$. The multi-faced structure is considered to be a non-equilibrium structure trapped as a result of the glass transition of PS occurring before the equilibrium state has been reached. Increasing the molecular weight of the polymer leads to a decrease of the rate of phase separation by spinodal decomposition (Bates 1991). A higher polymer molecular weight means that the glass transition can happen at higher solvent/non-solvent ratio at room temperature, which corresponds to an earlier stage of solvent exchange during the process (which might compensate for the decrease of the rate of solvent exchange due to the more viscous initial solution with larger polymer macromolecules). These might lead to the
polymer-polymer phase separation time becoming larger than the vitrification time of PS, which explain why the multi-faced structure is more likely to happen for polymers with higher molecular weight (in the specific experiment, about 100 times larger than for low molecular weight polymers). In addition, only Janus blend nanoparticles form at low initial polymer concentration and low PS/PI ratio. Multi-faced structures begin to appear and the nanoparticle sizes become larger with increasing initial polymer concentration in the case of higher PS/PI ratio. Increasing the fraction of PS and the concentration affects the details of the phase separation, such as the volume of confinement (particle size) and the viscosity, the fluctuation length scales etc., which might cause the glass transition of PS to happen before the complete phase separation of the polymers. While the hypothesis sounds plausible, the exact details are still not clear yet.

![Figure 3.1 Nanoprecipitation of PS/PI immiscible blend.](image)

The stability of the blend nanoparticles is achieved by the electrostatic stabilization mechanism, which is supported by a measured negative zeta potential. The surface charge is believed to come from the hydroxide ions of solution adsorbed onto the hydrophobic...
polymer surface since the homopolymers used do not carry ionizable end groups (Beattie, Djerdjiev et al. 2009).

Co-nanoprecipitation can be extended to different combinations of immiscible homopolymers or homopolymers with different end-groups under similar process (Sosa, Liu et al. 2016). Janus blend nanoparticles are realized in systems such as PS-PLA (poly (lactic acid)), PS-PB (polybutadiene) with and without hydroxyl end groups and PB-PLA, etc. Trials have also been carried out on systems with more than two homopolymers. In this case, tri-faced nanoparticles in nanoprecipitation system of three immiscible polymers PS, PB and PVCH (poly (vinyl cyclohexane)) were created.

3.1.2.2 Self-organized Precipitation (SORP)

Immiscible polymer blend nanoparticles were also formed applying the SORP method of nanoprecipitation in the work of Yabu’s group (Tajima, Higuchi et al. 2008, Motoyoshi, Tajima et al. 2010, Yabu 2013). The SORP method is a process of evaporating out good solvent of a near-saturated solution of solute/solvent/non-solvent to induce solute nanoprecipitation.

Figure 3.2 SEM and TEM images of PI/PMMA blend nanoparticles by SORP. (a) Hemispherical shaped blend nanoparticles formed at low polymer concentration; (b) spherical shaped blend nanoparticles formed at higher polymer concentration (c) TEM images of core-shell structure (PI core- PMMA shell). Reprinted from (Tajima, Higuchi et al. 2008). Copyright (2008), with permission from Elsevier.
As shown in Figure 3.2, core-shell structure blend nanoparticles of PI/PMMA (1/1 ratio) form in a THF/water system (without surfactants) upon THF evaporation over the course of 48 hours (Tajima, Higuchi et al. 2008). The core is composed of PI and the shell is PMMA. At low polymer concentration, blend nanoparticles are mostly hemispherical; with increasing polymer concentration, both the particle size and the width of the distribution increase and the particles transit from hemispheres to a combination of hemispheres and spheres and eventually to mostly spheres. In explaining the effect of concentration on particle shape, the article adopts a nucleation and growth mechanism. THF is a volatile-solvent and the evaporation happens at the air-solution interface. It is believed that the supersaturation level increases at a very slow pace upon THF removal and that the system gradually evolves from the near-saturated region into the metastable region. When the polymer concentration is low enough, phase separation by nucleation and growth occurs and ends at the air-solution interface to produce the hemispherical shape, based on observations of solution turbidity over time. At higher polymer concentrations, nucleation and homogenous growth of the particles can happen within the bulk solution to form spherical nanoparticles (Higuchi, Yabu et al. 2006). The nucleation and growth mechanism is also used to explain the core-shell structure of PI/PMMA (PI core and PMMA shell). The model relates the particle structure to the effect of the hydrophobicity of polymers on the rate of polymer phase separation in aqueous solution. Since PI is more hydrophobic than PMMA according to the solubility parameters reported (Brandrup, Immergut et al. 1989, Tajima, Higuchi et al. 2008), the PI phase separates from the solution faster than PMMA does, so the PI core forms first then PMMA precipitates onto the PI core as a shell. This proposed mechanism may be correct.
for this particular system due to the slow and gradual change in the SORP process, but the details are not fully understood and the model is only qualitative.

Various other immiscible polymer blend nanoparticles can be formed using SORP besides PI/PMMA, some with core-shell structure and others having Janus structure (See Figure 3.3) (Motoyoshi, Tajima et al. 2010). The author relates the final blend particle structure with the hydrophobicity of the polymers (affinity between polymer and water). Values of the solubility parameters ($\delta$) of polymers are used as an estimation of the hydrophobicity. As shown in Figure 3.3, it is claimed that if two homopolymers have similar solubility parameters, Janus blend nanoparticles form, but when their values are very different, the blend nanoparticles form core-shell structures with the more hydrophilic polymer as the shell. The prediction based on solubility parameters somewhat matches the experimental finding, but not fully. Many effects can cause this mismatch,
such as the limitations of solubility parameters to fully capture the interactions among the components as discussed in section 2.1.3.1.3.

To further support the hydrophobicity hypothesis for explaining the particle structure, blend particles were made with the thermo-responsive polymer TRP (Poly (N-isopropylacrylamide-co-N-dodecylacrylamide)) and PI by SORP under different temperatures. The hydrophobicity of TRP changes around the lower critical solution temperature (LCST), and the LCST of the particular TRP used is around 24°C (Motoyoshi, Tajima et al. 2010, Winnik, Davidson et al. 1992). When below LCST, the amido group of TRP can form hydrogen bonds with water so that the polymer becomes more hydrophilic; while above the LCST, the hydrogen bonds do not form due to the stronger thermal motion. It is found that when THF evaporation was carried out at 33°C (T> LCST), Janus blend nanoparticle formed. On the other hand, if the THF removal temperature was kept at 15°C (T< LCST), core-shell blend nanoparticles formed with PI as the core and TRP as the shell. This finding is consistent with the prediction of the hydrophobicity. Moreover, if the TRP/PI core-shell nanodispersion was annealed at 40°C (T> LCST) for a week, a structural transformation of the blend nanoparticles from core-shell to Janus was observed. The annealing process above LCST makes TRP become more hydrophobic, so it moves to reduce the contact area with water. Thermo-annealing was also carried out for the PI/PMMA core-shell blend nanodispersion, but no transition was observed, which suggests that the blend nanoparticles formed are at equilibrium since the SORP process is very slow and there is enough time to the particles to complete the polymer-polymer phase separation. This is a different circumstance compared to that
observed in the work of the Prud’homme group, in which the morphology of the blend nanoparticles was dependent on the kinetics.

3.1.3 Summary

In the work of both Prud’homme’s group and Yabu’s group, immisible polymer blend nanoparticles were realized by co-nanoprecipitation, and no pour homopolymer nanoparticles were found in any of the systems. The mechanism of the co-localization of different polymers relates to the lower interfacial energy between polymers than it is between polymer and solvents, but details are still under investigation. The formation mechanism might be spinodal decomposition with interfacial turbulence in the rapid mixing and high supersaturation condition of the systems investigated by Prud’homme, or nucleation and growth in the slow mixing and low supersaturation condition of the systems studied by Yabu. Both systems were surfactant free and the blend particle structure at equilibrium might be explained using the interactions among the materials. Since the polymers and the final particles are not covered with surfactants, the structural difference of the phase separation arises only from the interactions between polymers and between the polymer and the solvents (Motoyoshi, Tajima et al. 2010). Due to the nature of the experimental conditions, the glass transition temperature may also play a critical role in particle structure in Prud’homme’s work.

3.2 Nanoprecipitation of Charge Transfer Co-crystals

Inspired by the nanoprecipitation of single-component nanocrystals, some efforts have been made to test the feasibility of forming nano co-crystals by nanoprecipitation. This was first demonstrated in work of Nishida et al. in the formation of the charge transfer
complex perylene-TCNQ (tetracyanoquinodimethane) under fast mixing and high supersaturation condition (Van Keuren, Nishida 2010).

A binary charge transfer complex consists of two kinds of molecules with donor-acceptor interactions. In the perylene-TCNQ complex, TCNQ is the electron acceptor and perylene is the electron donor. Unique novel optical and electrical properties may arise that do not exist in individual components, which can lead to potential applications as organic semiconductors (Vermeulen, Zhu et al. 2014, Wu, Wang et al. 2013, Zhang, Geng et al. 2012, Nishida 2011). Perylene-TCNQ is one of the commonly studied binary charge transfer complexes and single crystals of perylene-TCNQ can be well synthesized, usually with sizes on the order of microns or even larger (Vermeulen, Zhu et al. 2014, Shokaryev, Buurma et al. 2008, Hu, Ma et al. 2014). Co-nanoprecipitation is a new approach in creating perylene-TCNQ and nanosize co-crystals have been observed (Nishida 2011). The process is carried out by injection of an initial mixture of perylene and TCNQ (1:1 molar ratio) in an acetone solution into pure water with moderate stirring (without surfactant). Nanoprecipitation of pure perylene and TCNQ were also done by the same process as control groups. The confirmation of co-crystal formation was done by optical measurement, primarily by absorption spectroscopy. The absorption spectrum of the co-crystal nanodispersion possesses a new absorption peak in the near infrared corresponding to the charge transfer state, which does not exist in pure perylene or TCNQ samples nor amorphous composites of perylene and TCNQ (Nishida 2011). This new absorption peak is broad and centered around 920 nm, which is believed to arise from the dimer of perylene and TCNQ. The formation of PT co-crystal is further confirmed by observed peak shifts from the pure components previously reported in
Raman spectra, which are due to the sensitiveness of vibration to the local environment change (Nishida 2011, Bandrauk, Truong et al. 1982). In addition, SEM images showed that nanoparticle shape of perylene-TCNQ differed from that of the perylene or the TCNQ. The growth behavior of the co-crystal was also investigated. The rapid appearance (within 1 second after mixing) of the new absorption peak was observed, which indicates the fast formation of the perylene-TCNQ dimer. This observation is consistent with the time scale of the initial molecular assembly as discussed previously, which can be within milliseconds. Time resolved DLS showed that the nano co-crystal continued to grow over a period of 30 mins, with the final nano crystals in the submicron size range and be stable for hours. Both the growth time and the final size of perylene-TCNQ are larger than for the pure perylene sample. The hydrodynamic radius of TCNQ from DLS cannot be determined due to the rapid formation of microcrystals as mentioned above.

The effect of two factors, the temperature and the molar ratio of perylene and TCNQ were also investigated in the work of Nishida et al. It was found that at high temperature (40℃), the 920 nm-absorption peak disappears and the particle size becomes smaller. At low temperature (7℃), co-crystals form while the particles keep growing for hours resulting in highly polydispersed samples. According to the author, the findings may relate to the dependence of the solubility of the solutes on temperature, which lead to formation of pure perylene and/or TCNQ crystals. For perylene and TCNQ with molar ratio 2:1, the 920nm absorption peak was observed but was slightly smaller than for perylene-TCNQ with 1:1 ratio, and the nanocrystals had smaller size. For perylene and TCNQ with molar ratio 1:2, the 920nm absorption peak also appeared and the particle
size was larger and more polydispersed. A possible explanation is that pure perylene or TCNQ crystals form with the excess amount of that component that was not incorporated into the co-crystal.

The study of Nishida et al. is preliminary and there are many questions remaining to be further investigated. One relates to the crystal structure of the perylene-TCNQ nano co-crystals formed. As reported in the literature (Figure 3.4), perylene (P)-TCNQ (T) can have two common crystal structures with 1:1 (P1T1) and 3:1 (P3T1) stoichiometric ratios, plus a recently reported 2:1 (P2T1) crystal structure under specific preparation conditions (Vermeulen, Zhu et al. 2014). Powder X-ray diffraction (PXRD) was carried out on freeze-dried perylene-TCNQ nanocrystals by Nishida, and the spectrum showed differences with the spectrum of pure perylene or TCNQ. However, the quality of the spectrum was not high enough to extract the exact crystal structure of the co-crystal. Besides, since no stabilizing agents are introduced in the perylene-TCNQ system, the stabilization mechanism of the co-crystals needs to be investigated. Other remaining questions relate to the co-crystal formation mechanism upon nanoprecipitation, the feasibility of expanding the co-nanoprecipitation method to form other charge transfer complex, etc.
3.3 Research Focus

In order to gain a better understanding of the formation of multi-component nanoparticles using nanoprecipitation, two multi-component nanoparticle systems were selected as model systems: immiscible blends of polystyrene (PS) and poly (methyl methacrylate) (PMMA); and the molecular co-crystal of perylene and TCNQ. In this work, the experiments and discussion of these two material systems will be separated in independent parts.

Co-nanoprecipitation of the immiscible polymer blend PS-PMMA together is one of the efforts to further test the capability of co-nanoprecipitation in typical stirred vessel set up. Trials studying the mechanisms of co-localization were also carried out. The ultimate goal is to bring new insights into co-nanoprecipitation, which in return would benefit the study of single polymer nanoprecipitation.

Research on a molecular co-crystal nano system is conducted as an expansion and continuation of the work done by Nishida (Nishida 2011). Among the questions listed above, some of them are addressed in my research. The crystal structure of the co-crystals formed is further investigated. The effect of molar ratio of perylene and TCNQ was reinvestigated to gain better understanding of the co-crystal formed. A discussion of possible formation and stabilization mechanisms is also provided.
4 CHARACTERIZATION METHODS

4.1 Dynamic Light Scattering

Dynamic light scatting (DLS) can be used to measure the particle size in dispersions and thereby monitor particle growth. The DLS apparatus primarily used in this research was assembled from individual components. The laser for excitation was a HeNe laser with wavelength of 633nm. The scattering signals were collected by a photodiode detector through a single mode optic fiber, which was placed at 90 degrees with respect to the incident laser. The scattered light was detected by an avalanche photo-diode single photon counting module (EG&G) and the signal was processed by an ALV5000 hardware autocorrelator (ALV GmbH, Germany). The particle dispersion right after nanoprecipitation was placed in a 3.5 ml glass cuvette and placed in the DLS apparatus. The refractive index and viscosity of the sample were set as parameters in the ALV5000 software depending on the sample. A typical time period used for size measurement was between 20 to 60 seconds. The signals were processed with either the ALV5000 software or by a MATLAB data-fitting program. These both output the mean value of size and the polydispersity index (PdI) for the hydrodynamic radius and its distribution. Both the size and the PdI were obtained based on a cumulants analysis of the intensity autocorrelation function generated by DLS. Multiple angle DLS was also carried out with DLS equipment from LS Instruments for polymer blend samples to confirm the particle size and size distribution. An additional description of the settings is also provided later in the experimental sections.
4.2 Electron Microscopies

Scanning electron microscopy (SEM) was used to imaging both the polymer blend samples and the charge transfer co-crystal samples. The equipment used was a Zeiss Supra 55VP. All the samples were measured using the secondary electron detector at 0.5 kV to 2 kV beam energy. Transmission electron microscopy (TEM) was also used to image the samples. TEM imaging was performed with a Philips EM400T microscope operated at 120 kV. All images were acquired with an SIS Cantega 2K CCD camera. Details of the sample preparation procedures can be found later in the experimental sections.

4.3 Absorption Spectroscopy

Absorption measurements were carried out on charge transfer co-crystal samples. The absorption spectra in the UV-VIS region were acquired with spectrometer “USB 4000” from Ocean Optics. The measurement range covers wavelength from 345 nm to 1037 nm. Spectra in the IR region were taken separately with TE Cooled InGaAs Array spectrometer from BWTEK Inc. The measurement range of this instrument covers wavelengths from 894 nm to 1700 nm. Detailed sample preparation procedures are described later in the co-crystal experimental section.

4.4 Powder X-ray Diffraction

Powder X-ray diffraction was used in order to determine the crystal structures of the charge transfer co-crystals. The equipment used was a Rigaku Ultima IV diffractometer with a Cu target. The voltage and current were set to 40 kV and 30 mA. The scintillation
counter was selected for the measurements. Detail sample preparation procedures are described later in the co-crystal experimental section.
5 EXPERIMENT – NANOPRECIPITATION OF PS-BLEND-PMMA

5.1 Sample Information

Polystyrene (PS) is one of the most common vinyl polymers used in industry in plastics and foams. Most commercially available PS polymers are atactic, in which the phenyl group randomly arranges itself at either side of the hydrocarbon backbone. Poly (methyl methacrylate) (PMMA) belongs to the family of vinyl polymers and is very common as well. The chemical structures of PS and PMMA are shown in Figure 5.1.

There are certain considerations upon choosing PS-blend-PMMA as a model system. First, PS and PMMA separately have commonly been used to study the nanoprecipitation process in polymers. Their nanodispersions have been shown to have particles with submicron size and good monodispersity, and a relatively good understanding of the systems has been achieved as reviewed in section 2.2.1 (Aubry, Ganachaud et al. 2009, Zhang, Pansare et al. 2012, Perevyazko, Vollrath et al. 2010, Perevyazko, Vollrath et al. 2012). In addition, both PS and PMMA have been studied as one of the components in immiscible polymer blend nanoparticle nanoprecipitation, such as PS/PI and PMMA/PI blend nanoparticles reviewed above (Sosa, Liu et al. 2016, Motoyoshi, Tajima et al. 2010). For PS and PMMA themselves, they are immiscible with each other and no study has been reported synthesizing them together by nanoprecipitation. The research on PS-blend-PMMA system can be better
understood based on previous work, it can also be used as a way to test the previously reported models and bring new insights.

5.2 Sample Preparation

5.2.1 Materials

All polymers used in the experiments were purchased from Scientific Polymer Products Inc, including PMMA, PS with various molecular weights and PbrS (Poly bromostyrene). The average polymer molecular weights of PS were 18,100 g/mol, 48,100 g/mol, 75,500 g/mol or 100,000 g/mol (dicarboxy terminated) respectively. The PbrS had a molecular weight of 60,000 g/mol. PMMA used for blend nanoprecipitation was in the form of white, fine beads and had an average molecular weight of 75,000 g/mol. PMMA with a higher molecular weight (540,000 g/mol) was used once as a morphology test for PMMA nanoprecipitation. THF was chosen as a good solvent for the nanoprecipitation process and was purchased from Fisher Scientific. Water or NaOH aqueous solution was used as miscible non-solvent. Both NaOH pellets and HPLC water were purchased from Fisher Scientific. All materials were used without further purification.

5.2.2 Nanoprecipitation Process and Sample Groups

5.2.2.1 Typical Procedure

A typical nanoprecipitation procedure employed in this research is described as follows. Initial polymer solutions PS/THF and PMMA/THF were prepared by separately dissolving PS and PMMA into THF overnight. PS easily dissolves in THF while it takes several hours for PMMA to fully dissolve in THF at room temperature. Equal portions of
PS/THF and PMMA/THF were mixed together to make the initial PSPMMA/THF solution. NaOH aqueous solutions were prepared by dissolving a small amount of NaOH into HPLC-grade water and the pH value was kept between 9 and 10. The nanoprecipitation was carried out by injecting the initial polymer solution into a water or NaOH aqueous solution at a solvent/non-solvent volume ratio of 1/9. The injection was automated using a Hamilton Microlab 500 syringe pump. The apparatus consists of two separate channels, left and right. Each channel consists of one inlet, one automated syringe and one outlet, as shown in the schematic in Fig 5.2. Typically, only one channel was used. The inlet connects to the reservoir of initial polymer solution at one end and to the syringe pump on the other end. Through the inlet, the syringes can be automatically filled with initial polymer solutions. The outlet connects to the syringe as well at one end and the other end is inserted in the reservoir of the non-solvent (usually glass bottles). The non-solvent is constantly magnetic stirred for a few minutes during the mixing process. The initial polymer solution in the syringe can be automatically injected into the non-solvent at various injection rates (such as 0.2 ml/s). All the experiments were preformed at room temperature. Variations of the process performed in this research will be described in the specific sample groups listed as follows.

Figure 5.2 Automatic injection system of PSPMMA co-nanoprecipitation. (a) Schematic diagram of the experimental set-up with automatic injection; (b) Hamilton Microlab 500 syringe pump used for automatic injection.
5.2.2.2 Sample Groups

5.2.2.2.1 “PS (48,100) PMMA and Sequential Injection” Samples

The “PS (48,100) PMMA” sample series contains six samples: they are four normal nanoprecipitation samples “PS (48,100)-blend-PMMA”, “PS (48,100)”, “PMMA”, “PS (48,100)+PMMA” and two sequential injection samples “PS (48,100)-60s-PMMA” and “PMMA-60s-PS (48,100)”. For “PS (48,100)-blend-PMMA”, “PS (48,100)” and “PMMA”, each sample only use the right channel of the syringe pump to inject 1ml initial polymer solution into 9 ml NaOH aqueous solution. For “PS (48,100)-blend-PMMA” (or “PS(48,100)PMMA”), the initial PSPMMA/THF solution was prepared by mixing equal amount of $1.33 \times 10^{-5} \text{ mol/L}$ PS/THF solution (0.64 mg/ml) and PMMA/THF (1mg/ml) solution, with the resulting molar concentration of both PS and PMMA in THF equal to $6.67 \times 10^{-6} \text{ mol/L}$. “PS (48,100)” and “PMMA” of pure polymer nanoprecipitations were carried out as control trials, with the initial concentration of PS/THF or PMMA/THF equal to $6.67 \times 10^{-6} \text{ mol/L}$. “PS (48,100)+PMMA” is made simply by mixing equal amount of “PS (48,100)” and “PMMA” final nanodispersions, and was used to test the size measurement quality of DLS. In addition, to get some insight into the possible formation mechanism, sequential injections of PS (48,100)/THF solution and PMMA/THF solution into NaOH aqueous solution were also carried out as control trials. Both left and right channels of the syringe pump were used for sequential injection. The notation “PS (48,100)-60s-PMMA”(or “PS-PMMA”) stands for first injecting 0.5 ml PS/THF ($1.33 \times 10^{-5} \text{ mol/L}$) from one channel
of the syringe pump into a 9 ml NaOH aqueous solution, waiting for 60s and then injecting 0.5 ml PMMA/THF of the same concentration into the same solution through the other channel. “PMMA-60s-PS (48,100)” (or “PMMA-PS”) sample was prepared likewise, just by reversing the injection sequence. For these samples, the injection rate of the initial polymer solution was set at 0.2 ml/s. NaOH aqueous solutions with a molar concentration of 0.1 mM (degased with nitrogen) were used as the non-solvent, with the pH value kept around 9.9. Although the molar concentration of NaOH was higher than the polymer concentrations in all the samples, the value was still very low and the pH value decreased noticeably over time due to the reaction with carbon dioxide in the air. The NaOH aqueous solution was degassed with nitrogen gas to maintain its pH value before nanoprecipitation. The non-solvent was constantly stirred at a rate of 1200 rpm during and after nanoprecipitation for a total of 2 minutes. A solvent removal process was performed 30mins after the nanoprecipitation. Half of the sample solutions was stirred under a fume hood for 3.5 hours or more to remove the THF. Typically, a 5 ml sample was reduced to 3.2 ml after the THF removal process.

5.2.2.2.2 “PS (MW)-blend-PMMA and PbrS-blend-PMMA” Samples

PS of various molecular weights and PbrS were used to make blend nanoparticles with PMMA (molecular weight of 75,000 g/mol). The sample notations “PS (18,100) PMMA”, “PS (48,100) PMMA”, “PS (75,700) PMMA” and “PbrS (60,000) PMMA” stand for the blend samples using PS with molecular weight of 18,100g/mol, 48,100 g/mol, and 75,500 g/mol respectively and PbrS with a molecular weight of 60,000g/mol. The concentrations used, the nanoprecipitation process and the following procedures were all same as the PS(48,100)PMMA sample.
5.2.2.2.3 “PS (75,700) PMMA Manual Injection” Samples

This set of samples includes PS (75,000) PMMA blend samples at two concentrations, pure PS (75,000) samples and pure PMMA samples of two molecular weights, sequential injection samples PS-60s-PMMA and PMMA-60s-PS, and a PS+PMMMA sample. One PSPMMA blend sample (labeled as “PSPMMA1”) has molar concentrations same as the PS (48,100) PMMA series. The other PSPMMA blend sample (labeled as “PSPMMA0.5”) has half of the molar concentration of PSPMMA1. A trial of nanoprecipitation was also carried out on pure PMMA with a higher molecular weight (540,000 g/mol). The nanoprecipitation process was carried out by manual syringe injection of a 2 ml initial solution in roughly 5 seconds into an 18 ml NaOH aqueous solution. The solution was magnetically stirred during and after the injection process for 2 minutes at a rate of 600 rpm. All the other experimental conditions, and concentrations were the same as the PS (48,100) PMMA series, except the mixing details.

5.2.2.3 Sample Characterization

5.2.2.3.1 DLS measurement

Sample growth was monitored for the samples starting 3 minutes after nanoprecipitation and continuing for 30mins, using a DLS instrument built from components. The samples (both original and THF-removed) were also measured later at 4 to 6 hours and 1 day after nanoprecipitation. Multiple angle DLS was also carried out on 1-day old THF-removed samples using the DLS apparatus from LS Instruments. The measurement angles were set from 20 degree to 130 degree with a 10-degree sampling interval. For both original samples and THF-removed samples, the refractive index and viscosity of the medium
were set to values of water, with the refractive index 1.33 and viscosity 0.89 cp. With the ratio of THF over NaOH aqueous solution at 1 over 9, the change of refractive index and viscosity is minor and does not significantly affect the calculated particle size.

5.2.2.3.2 TEM and SEM sample preparation

THF-removed 1-day old nanodispersions were used for TEM and SEM imaging, unless otherwise specified. Both drop-casting and spray-drying methods were used for TEM sample preparation. Drop-casting was done by adding a 15 μl drop of sample onto the copper grid, waiting for 10 minutes, and wicking dry the extra solution with filter paper. The spray drying procedure was done by directly spraying sample solutions onto the copper grids one or two times. TEM samples were vapor-stained by 5% of ruthenium tetroxide (RuO₄) aqueous solution for 4 hours in order to enhance imaging contrast. Trent et al. found PS can be stained by RuO₄ vapor while PMMA is not affected (Trent, Scheinbeim et al. 1983). If the blend particles were not stained enough, structural changes upon long time electron beam bombarding were observed. For SEM samples, preparation methods included drop-casting, freeze-drying and spray-drying. Drop-casting was done by dropping a 50 μl final dispersion onto the aluminum stub and leaving it to dry in the fume hood. Freeze-drying was achieved by two approaches. One approach was lyophilizing a 5 ml sample in a glass tube, which was done by first quickly freezing the sample in liquid nitrogen and then leaving it in the freeze dryer for several days (in vacuum and below 0°C). When freeze-drying was done, the blend particles were re-dispersed into pure water by sonication and then the re-dispersed particle solutions were dropped onto the aluminum stub and let it dry. Another approach was directly lyophilizing a drop of sample on a stub, which was done by freezing the sample drop on
the stub in a freezer for 30 minutes and leaving it in the freeze dryer. To minimize the sample aggregation, a spray-drying method was also used. The samples were sprayed carefully several times onto the sample stub and left to dry.

5.3 Experimental Results

5.3.1 PS (48,100) PMMA Series and Sequential Injection

As shown by TEM images of the 1-day old THF-removed samples (spray dried) in Figure 5.3, nanoparticles of the PS (48,100) PMMA blend have a core-shell structure and are distinct from the nanoparticles of the control groups. The PS nanoparticles are large clean spheres and are free from the wrapping-like structure. The PMMA nanoparticle have various irregular shapes: most of them are network structures containing bubbles (the light contrast), while some of them are aggregates of very small particles or irregular large particles. For PSPMMA, the blend nanoparticles consist of darker cores with and lighter shells, which seem to be aggregated together. The shell is believed to be PMMA, while the composition of the core can be either mostly PS or well mixed PS and PMMA. It can also be seen that the core of the blend nanoparticles is not as spherical or as large as the pure PS nanoparticles. None of the structures in the pure PS and PMMA samples resemble the core-shell structure of the blend nanoparticles. This observation serves as a key evidence of the blend nanoparticle formation.
Figure 5.3 TEM images of spray-dried PS (48,100) PMMA samples. All the samples are 1-day old and THF removed. (a) and (b) for PS (48,100) PMMA, (c) and (d) for PS (48,100); (e) and (f) for PMMA.
The morphology of the blend nanoparticles is also different from the “PS+PMMA” as shown in Figure 5.5 (a)-(c). In these samples, the PS nanoparticles are connected by PMMA network structures and both the PS and PMMA parts are similar to those in the pure samples. This rules out the likelihood that the core-shell structure of the blend nanoparticles forms during the spray drying process. Moreover, it can be seen that the blend particles already form before the THF removal process as indicated by the TEM image (Figure 5.4) of PSPMMA at 30 minutes after nanoprecipitation. The PSPMMA nanoparticles at 30 minutes already have a similar particle size and core-shell structure as the 1 day-old THF-removed sample. In conclusion, these two observations suggest that the blend nanoparticles form during the nanoprecipitation process.
Figure 5.5 TEM images of “PS+PMMA” sample and the sequential injection samples. The samples were all spray dried. (a)-(c) are “PS+PMMA”, (d)-(f) are “PS-60s-PMMA”, (g)-(i) are “PMMA-60s-PS”. The TEM samples were prepared under the same conditions and techniques as the samples in Fig 5.4.

For sequential injection samples, the TEM images are shown in Figure 5.5. The PS-PMMA and PMMA-PS (injection waiting time 60s) samples look similar and there is no obvious feature to differentiate them from these images. Both of them are different from the blend sample, with the PS part similar to the pure PS nanoparticles and the PMMA part in the form of a continuous network. This finding suggests that a good portion of separate PS and PMMA particles form during the sequential injection. But it does not
rule out the possibility that there might be small portions of the blend nanoparticles formed in these samples that not detected in the images. It will be shown later in PS(75,700)PMMA manual injection samples that there is a small amount of blend nanoparticles present in the TEM images of the PMMA-PS sequence injection sample.

Particle aggregation is a common phenomenon of the TEM images in this research, except in the case of the pure PS samples. It is not only observed here but also in later sample groups. Pure PS particles keep their own spherical shape even when they aggregate (Figure 5.3(d)). For PSPMMA blend samples, nanoparticles in contact form into aggregates and the PMMA parts fuse together and evenly wrap around the cores. The fusion of PMMA is commonly observed in TEM images of all samples with a PMMA component. For instance, network structures of PMMA are observed in TEM images of pure PMMA, PS+PMMA, PS-PMMA and PMMA-PS samples (Figure 5.4-5.6, Figure 5.8). These aggregations mainly occur during the drying process according to the size information obtained by DLS given in Table 5.1 of the nanodispersions corresponding to the TEM samples. The particles in the nanodispersions (1day old, THF removed) have average hydrodynamic radius in the submicron region and moderate polydispersity. Both the average size and polydispersity by DLS well match the pure nanoparticles within the aggregates in the TEM images. If severe aggregation already existed in these nanodispersions, the intensity of scattered light would be dominated by the large aggregates and DLS would yield much larger particle size and polydispersity. The fusion of PMMA upon drying may be due to the properties of the PMMA in the dispersion such as its glass transition and solubility in water. Unlike the PS that maintains a spherical shape upon drying, the PMMA nanoparticle or shell is flexible enough to form a
A continuous structure. Using the blend sample as an illustration, several particles within one spray droplet come together upon drying and the PMMA shell of the blend particles fuse together. The level of severity of particle aggregation varies in the TEM image. This is because the spraying is done manually and the nozzle needs to be washed and re-adjusted after each use, making it hard to be consistent with the nozzle adjustment, so the spraying droplet size and spray duration can vary every time. The bubble like structure of PMMA may relate to the spray drying process, since it is only observed in samples prepared by spray drying, not in samples by drop casting, as will be later discussed in “PS (75,000) PMMA series”.

<table>
<thead>
<tr>
<th>Blend Samples</th>
<th>4h -SolY</th>
<th>1D -SolY</th>
<th>4h -SolN</th>
<th>1D -SolN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS(48,100)PMMA</td>
<td>88±3 (0.11±0.02)</td>
<td>91±2 (0.12±0.04)</td>
<td>58±1 (0.10±0.01)</td>
<td>64±1 (0.09±0.04)</td>
</tr>
<tr>
<td>PS(48,100)</td>
<td>111±9 (0.10±0.03)</td>
<td>103±23 (0.16±0.08)</td>
<td>90±2 (0.07±0.03)</td>
<td>91±1 (0.11±0.05)</td>
</tr>
<tr>
<td>PMMA</td>
<td>100±1 (0.23±0.05)</td>
<td>366±20 (0.83±0.02)</td>
<td>37±1 (0.15±0.05)</td>
<td>38±1 (0.14±0.02)</td>
</tr>
<tr>
<td>PS(48,100)-PMMA</td>
<td>125±4 (0.11±0.07)</td>
<td>124±3 (0.08±0.05)</td>
<td>94±2 (0.15±0.04)</td>
<td>97±1 (0.14±0.04)</td>
</tr>
<tr>
<td>PMMA-PS(48,100)</td>
<td>119±2 (0.14±0.03)</td>
<td>117±2 (0.08±0.03)</td>
<td>91±1 (0.13±0.04)</td>
<td>92±1 (0.09±0.03)</td>
</tr>
<tr>
<td>PS(48,100)+PMMA</td>
<td>N</td>
<td>N</td>
<td>87±1 (0.11±0.03)</td>
<td>N</td>
</tr>
</tbody>
</table>

| Table 5.1 Hydrodynamic radius (nm) and PDI of PS (48,100) PMMA samples. The size information is based on the same set of samples as for TEM imaging above. Particle size refers to hydrodynamic radius. Original (-SolY) samples and solvent removed (-SolN) samples were measured with DLS at 4 hour and 1 day respectively. The values were calculated based on 5 runs of measurements. |

Particle growth in the same set of samples (as for TEM imaging) was monitored overtime by DLS from 3 to 30 minutes and later at 4 hours and 1 day after nanoprecipitation, as shown in Figure 5.6 and Table 5.1. The particles underwent significant growth within the first 3 minutes of nanoprecipitation before DLS measurement. Over the time scales of the DLS measurements, the particles continued to grow, but much slower. All samples at 30 minute produced nanoparticles in the low submicron range and are moderately polydisperse according to the PDI values. The PMMA nanodispersions were comparatively most polydisperse. PS was most stable and no flocculation was observed.
for months. The PMMA original sample was least stable and flocculation was observed within one day after nanoprecipitation. The PSPMMA original sample was in between, maintaining stability for several days. Meanwhile, the THF-removed nanodispersions all had smaller particle sizes and were more stable compared to the samples without THF removal. However, precipitates did occur in PMMA and PSPMMA THF removed samples within a few weeks.

![Figure 5.6](image)

**Figure 5.6 Particle growth of PS (48,100) PMMA samples from 3 to 30 minutes.** The size information is based on the same set of samples as for TEM imaging above.

For the sequential injection samples PS-PMMA and PMMA-PS, both of their particle size behaviors resembled the pure PS sample based on DLS measurements. The particle growth data and later particle size and distribution were similar to that of the pure PS sample. It is believed that this observation is due to the formation of considerable amounts of pure PS particles in both dispersions of PS-PMMA and PMMS-PS. The bigger PS particles dominate the scattering signal of DLS. This claim is supported by the TEM images and previous discussions. The images of particles in PS-PMMA and
PMMA-PS resemble each other, which consist of larger spherical PS particles and the PMMA network. They look very different from the PSPMMA blend samples and the pure PS sample. This observation also suggests the limitation of DLS, which is further confirmed by the “PS+PMMA”. “PS+PMMA” samples, which are mixtures of THF removed PS sample and PMMA sample, are also confirmed in the TEM images (Figure 5.5). However, these have size and PDI very similar to the pure PS as shown in column “1D-SolvN” of Table 5.1. This suggests the scattering intensity detected by DLS in “PS+PMMA” is mainly from larger PS particles and the size information of PMMA particles cannot be resolved from the DLS measurement. The quality and reliability of this data was confirmed with the LS instrument, as shown in Figure 5.7. Multiple angle DLS measurement was carried out for the samples (1day old, THF removed), and the resulting size information agrees well with the data in Table 5.1.

![Figure 5.7 Multi-angle DLS of PS (48,100) PMMA samples](image)

**Figure 5.7 Multi-angle DLS of PS (48,100) PMMA samples.** The measurement was carried out by LS instrument. Angles of measurement are from 20 to 130 degree, with interval of 10 degrees. The size information is based on the same set of samples as for TEM imaging above.

The size variance between identically prepared samples (sample repeatability) is another concern for PSPMMA and PMMA nanoprecipitation, based on particle size comparison between repeated trials and the occurrence of flocculation in original samples or upon
THF removal. The study of reproducibility was mainly based on PS(75,700)PMMA manual injection samples, and it will be discussed in details later. It was found that the sizes of nanoparticles vary among trials and occasionally the dispersions become unstable during the THF removal process and flocculation occurs. For automatic injection samples, the reproducibility improved comparing to manual injection samples but this issue was not completely resolved. For instance, one PS (48,100) PMMA blend sample prepared with injection rate at 1ml/s was stable 4 hours after nanoprecipitation and had particle size (original) around 120 nm; while in another trial with same experimental condition, precipitation occurred.

5.3.2 PS (different MW) PMMA Series and PbrSPMMA

PSPMMA with PS of different molecular weights and PbrSPMMA blend samples were also prepared. The same experimental conditions and molar ratios were used as in the PS (48,100) PMMA series. As shown in Figure 5.8 and Table 5.2, the blend nanoparticles are of submicron particle size and have moderate polydispersity. The morphology of the blend particles all resembles the PS-core-PMMA-shell structure. This further suggests the formation of PSPMMA blend nanoparticles with the nanoprecipitation method.
Figure 5.8 TEM images of PS (different MW) PMMA blend particles. All the samples were 1 day old and THF removed. The TEM samples were prepared under the same conditions by the spray-drying method as samples in Fig 5.4. (a) PS (18,100) PMMA, (b) PS (48,100) PMMA, (c) PS (75,000) PMMA and (d) PbrS (60,000) PMMA.

Aggregation of the blend nanoparticles in the TEM images was also observed as described previously due to the fusion of PMMA during the drying process. Among the samples, the blend nanoparticles of PS (18,100) PMMA have the smallest core and the most obvious PMMA shell. For PS(48,100)PMMA and PS(75,000)PMMA, the PMMA shell is much thinner since their core is much larger. But the exact thickness of the PMMA shell could not be measured accurately enough to perform any quantitative calculation. The thin shell structure of PMMA is also observed in PI-PMMA blend
sample reported, and PI-PVAc and PI-PMA also have similar structures (Figure 3.3) (Motoyoshi, Tajima et al. 2010). The comparatively core of PS (18,100) PMMA may suggest that shorter PS chains would lead to a smaller size of the core in blend nanoparticles, considering the polymer molar concentrations were kept the same in all blend samples. The polymer chain of PS is shorter with a lower molecular weight, so the core is smaller when a similar number of PS chains are entrapped in the core area and polymer-polymer phase separation between PS and PMMA may be faster, which also contribute to the core size reduction. In Table 5.3, the number of monomers (length of the chain) in each polymer chain is roughly calculated by dividing the polymer molecular weight with the molar mass of monomers. The molar mass of styrene is about 104 g/mol and the molar mass of bromostyrene is about 183 g/mol. It predicts that the sizes of the blend samples and the cores follow the relation PS (18,100) PMMA < PbrS (60,000) PMMA < PS (48,100) PMMA < PS (75,700) PMMA. The DLS data and TEM imaging of the blend samples are in accordance with the relation. However, the evidence here is insufficient to support this claim, since both the size variance issue and the moderate size distribution observed in TEM images of the samples hamper the information extraction about the effect of MW on particle size. For example, 4-hour old original sample of PS(18,100)PMMA in another repeating trial had size around 89 nm instead of the 50 nm value shown in Table 5.2. In addition, the effect of the bromine atom of PbrS on particle formation behavior is also a factor to investigate.

All blend particles have a particle growth burst within 3 minutes of nanoprecipitation and grow slowly after that. Blend nanodispersions with THF removed have smaller particle
sizes and are more stable than the original blend samples. Overall, the particle behaviors of the blend samples agree with the findings in PS (48,100) PMMA series.

<table>
<thead>
<tr>
<th>Blend Samples</th>
<th>4h-SolY</th>
<th>1D-SolY</th>
<th>4h-SolN</th>
<th>1D-SolN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS(18,100)PMMA</td>
<td>50.4±0.6 (0.9±0.05)</td>
<td>58.0±1.5 (0.12±0.04)</td>
<td>35.7±0.5 (0.11±0.03)</td>
<td>37.2±0.4 (0.10±0.01)</td>
</tr>
<tr>
<td>PS(48,100)PMMA</td>
<td>87.7±3.5 (0.11±0.02)</td>
<td>90.5±1.6 (0.12±0.04)</td>
<td>58.5±1.0 (0.10±0.01)</td>
<td>64.2±0.7 (0.09±0.04)</td>
</tr>
<tr>
<td>PS(75,700)PMMA</td>
<td>85.9±10.0 (0.13±0.03)</td>
<td>94.9±2.3 (0.09±0.04)</td>
<td>67.5±0.9 (0.11±0.04)</td>
<td>72.2±1.0 (0.11±0.03)</td>
</tr>
<tr>
<td>PbrSPMMA</td>
<td>129.9±8.3 (0.27±0.25)</td>
<td>Precipitates</td>
<td>55.0±0.4 (0.08±0.03)</td>
<td>58.0±0.4 (0.09±0.02)</td>
</tr>
</tbody>
</table>

Table 5.2 Hydrodynamic radius (nm) and PDI of PS (MW) PMMA samples. The size information is based on the same set of samples as for TEM imaging above. Both original (-SolY) samples and solvent removed (-SolN) samples were measured by DLS at 4 hour and 1 day respectively. The values were calculated based on 5 runs of measurements.

<table>
<thead>
<tr>
<th>PS (Molecular weight g/mol)</th>
<th>Molar mass of monomer (g/mol)</th>
<th>Number of monomers in each polymer chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS (18,100)</td>
<td>Styrene (104)</td>
<td>174</td>
</tr>
<tr>
<td>PS (48,100)</td>
<td></td>
<td>463</td>
</tr>
<tr>
<td>PS (75,700)</td>
<td></td>
<td>728</td>
</tr>
<tr>
<td>PbrS (60,000)</td>
<td>4-bromostyrene (183)</td>
<td>328</td>
</tr>
</tbody>
</table>

Table 5.3 Estimated number of monomers of PS polymer chain with different molecular weights. The number of monomers of PS polymer chain is estimated by dividing molecular weight by molar mass of monomer.

5.3.3 PS (75,700) PMMA Manual Injection Samples

As shown by the TEM images in Figure 5.9, the manual injection samples of PS (75,700) PMMA, pure PS and PMMA have similar particle morphology as the automatic injection samples. The PS nanoparticles are spherical, with sizes below 200nms and moderate size distribution. For PMMA, irregular shaped continuous aggregates dominate the TEM images. The PSPMMA blend nanoparticles have PS-core-PMMA-shell structure and their PMMA part leads to aggregation. It can also be seen that both TEM preparation methods, drop-cast/wick dry and spray dry, gave similar quality of images. The aggregation problem in the drying process exists in both methods. A difference in bubble structures appears in the spray-dried PMMA sample but not in drop-cast samples. Unlike
Figure 5.9 TEM images of PS (75,700) PMMA manual injection samples. (a), (c) and (e) are TEM images of pure PS(75,700), PMMA and PS(75,700)PMMA blend samples (THF-removed) prepared by drop-casting. The drop-casting was done by letting a drop of nanodispersion sit on the copper grid for 10 minutes and then being wicked dry; (b), (d) and (f) are TEM images of same samples prepared by spray-drying.
the PMMA sample, images of PSPMMA for both drying methods are free from bubble like structures. It is unknown why the bubble structure appears in spray-dried PMMA samples but not in spray dried PSPMMA samples. These bubble structures upon spray drying may relate to the free pure PMMA nanoparticles and their characteristics during the spray drying process since other samples with free pure PMMA nanoparticles also have these structures. For instance, the “PS (48,100)+PMMA” sample shows obvious bubble structures and the sequential injection samples also have minor ones, as seen in Figure 5.5.

**Figure 5.10 TEM images of PMMA-PS (75,700) sequential injection sample.** The sample has THF removed and was prepared by drop-casting. (a) nanoparticles with core-shell structure; (b) PS nanoparticles connected by the PMMA network.

**Figure 5.11 Sample images of PSPMMA dispersions.** (a) and (b) are PSPMMA 1day-old original samples and THF-removed samples respectively: (1) PS; (2) PMMA; (3) PSPMMA0.5; (4) PSPMMA1; (5) PS-PM; (6) PM-PS. Dispersions of PSPMMA and PMMA samples with precipitates are also presented.
Typically, the samples appear as shown in Figure 5.11. The PS nanodisperion is turbid, while the PMMA nanodispersion is mostly clear, and the blend nanodispersion is in between. DLS measurements were also performed on manual injection samples.

The particle size behavior resembles the automatic injection samples. Significant particle growth seems to occur before the DLS measurements are started, and later the pace of growth slows down. Size variance occurs in all samples while flocculation only happens in PMMA or PSPMMA samples. Using the original samples at 30-minute as illustration, the PS nanoparticles had average radius ranging from 118 nm to 145 nm among trials and the final PS nanodispersions were very stable and did not precipitate for months. While in trials of PMMA, particles had average size ranging from 45 nm to 135 nm. The average sizes of PSPMMA 0.5 varied from 88nm to 138nm among trials and the average sizes of PSPMMA 1 were from 86 to 116 nm. The flocculation of the pure PMMA samples was usually observed within 1 day or 2 and the flocculation of PSPMMA happens within a week, as shown in Figure 5.11. Samples in most trials remained stable during the THF removal process and the resulting THF-removed nanodispersions were very stable and had moderate polydispersity. However, severe aggregation happened much faster within the dispersion sometimes even when the experimental conditions were kept the same. In some cases, much larger sizes were detected in PMMA or PSPMMA samples at early stages of nanoprecipitation and flocculation occurred upon THF removal. The size repeatability issue hinders the study of the effect of concentration on particle size. We were unable to compare the particle size of PSPMMA0.5 and PSPMMA1 as they vary in similar range. For particle morphology, the PSPMMA blend samples had good reproducibility and the nanoparticles always had similar core-shell structure in TEM.
images even when the particle sizes measured in DLS were different. Hence, the cause of the size variation of PMMA and PSPMMA and their tendency to flocculate may be due to the aggregation of PMMA nanoparticles or the PMMA shells of blend nanoparticles. A more detailed hypothesis is laid out in section 5.4.

Different drying processes were tried to prepare samples for SEM imaging. In the drop cast-air dry method, no aggregation was seen in the pure PS samples. Meanwhile, significant film and network formation was observed in PMMA and PSPMMA blend samples as shown in Figure 5.12. There were three different appearances for the PMMA sample. The images (b) and (c) contain aggregates of irregular particles covered by a continuous film, they are observed near the edge of the dried solution droplet. The image (d) shows very small nanoparticles connecting by networks, this structure is most widely observed in the middle region of the droplet. PMMA with higher molecular weight (540,000 g/mol) was prepared to compare with the PMMA (75,000 g/mol) sample. It was found that their structures resemble each other, as shown by the SEM images (drop cast-air dry) in Figure 5.13. Both the film and the network structures appear in the PMMA (540,000 g/mol) sample. For PSPMMA0.5 and PSPMMA1, they share a similar look, that is, a structure of uniformly dispersed nanoparticles connected by continuous film and network. The observation is consistent with the appearance of TEM images of samples prepared by the drop cast-wick dry method or the spray dry method, but the aggregation is much more severe. This further confirms the PMMA fusion upon drying and the flexibility of PMMA in the nanodisperions.
Figure 5.12 SEM images of PS (75,700) PMMA samples (drop cast-air dry). (a) PS; (b)-(d) different structures of PMMA; (e) PSPMMA 1; (f) PSPMMA 0.5.
Figure 5.13 SEM images of PMMA (540,000 g/mol) sample (drop cast-air dry). (a) film structure (b) network structure.

Freeze drying methods were also tested, initially to minimize the effect of the drying process on particle morphology and to keep the nanoparticles in their original form as in the solution. In Figure 5.14, two structures are found in samples of freeze-drying directly on the stub. One is similar to the spray dry samples, with nanoparticles scattered on the stub; another is with thin blocks of particle aggregates. For samples prepared by freeze-drying and redispersing, thin sheets of particle aggregates are observed, with small nanoparticles scattered in the polymer sheets. The block or sheet structures are also observed in PMMA samples. The cause of the formation of these structures are not clear, it might relate to the glass transition of PMMA during the freeze-drying process.
Figure 5.14 SEM images of PSPMMA and PMMA samples by freeze-drying. (a) and (b): PSPMMA prepared by freeze drying on stub; (c): PSPMMA prepared by freeze drying and redispersing; (d) and (e): PMMA prepared by freeze drying on stub; (f): PMMA prepared by freeze drying and redispersing.
5.4 Discussion

5.4.1 Analysis of Experimental Findings

In this study, I demonstrated the formation of PSPMMA blend nanoparticles by nanoprecipitation in a stirred vessel. The blend samples have core (PS) – shell (PMMA) structure in the TEM images, which are different from the pure PS or PMMA samples. This claim is further supported by the differences of their sizes as measured using DLS, and their stability in the nanodispersions. This study also shows that the blend formation happens during the nanoprecipitation process rather than later during treatment of the samples. First, the appearance of the PSPMMA blend sample in TEM images significantly differs from that of the “PS+PMMA” control sample; this observation rules out the possibility of core-shell structure formation during the spray drying process. From the TEM images of the PSPMMA original sample at 30 minutes, one can further conclude that the blend nanoparticles form before the THF removal process, since the nanoparticles observed are already of the core-shell structure. This point is supported by the size difference between samples of PSPMMA and PS or PMMA as early as 3 minutes after nanoprecipitation. Additional small numbers of blend nanoparticles may form during the THF removal process from dissolved PS and PMMA macromolecules. We are unable to determine whether small amounts of pure PS or PMMA nanoparticles form in blend nanoprecipitation based on current observations by TEM and DLS.

Large-scale film and network structures were observed in TEM and SEM images of PMMA and PSPMMA samples, and it is believed to be due to PMMA fusion upon various drying processes. This observation indicates that the outer PMMA layer of the
PSPMMA or PMMA nanoparticles in the nanodispersions is in a rubbery state at room temperature and those PMMA chains have good mobility at the PMMA/water interface. At some point during the drying process, the outer PMMA layers fuse together. Meanwhile, there is no aggregation problem observed for PS images upon drying, the nanoparticles retain individual spherical shapes even in contact with each other. This indicates that the surface of PS nanoparticles in the final dispersion is still in the glassy state and the polymer chains are rigid. Note that the glassy or rubbery state relates to the idea of glass transition. The nanoparticles in this study are all in the amorphous state so that they can go through glass transition. If ambient temperature (in our study, it is room temperature RT) is larger than glass transition temperature (Tg) of a polymer, the polymer is in rubbery state, and if the opposite, the polymer is in glassy state. For PS and PMMA, their bulk glass transition temperatures $T_{g,bulk}$ are reported both near 378 K (105°C), so they should be in glassy state at room temperature (Askadskiĭ 2003). However, Tgs of polymer nanoparticles in solution can be smaller than their $T_{g,bulk}$ due to the Tg confinement effect (Zhang, Guo et al. 2011). Zhang et al. measured the Tg of PS nanoparticles (spherical in SEM images) suspended in aqueous solution with modulated DSC. As shown in Figure 5.15 (Zhang, Guo et al. 2011), Tg values for PS nanoparticles are size dependent and the value can decrease to 320 K (46°C) at a hydrodynamic diameter of 90 nm. To explain the Tg confinement effect, the author applies a gradient model. At the particle free surface (here is the PS/water interface), the mobility of PS polymer chains is enhanced, causing a Tg reduction at the interface ($T_{g,surf}$). The Tg reduction effect penetrates into the interior the nanoparticle and the Tg gradually increases back to $T_{g,bulk}$. The smaller the size of the nanoparticle, the larger
ratio of interface area over volume, which leads to a more obvious Tg reduction measured experimentally. A good fitting to data is achieved based on this model when $T_{g,\text{surf}}$ is set to 305K (32°C) (Papaleo, Leal et al. 2006, Zhang, Guo et al. 2011). The penetration layer is found to be around 30 nm and it agrees well with the studies on PS thin films. Here it assumes that the Tg depends only on nanoparticle size but not molecular weight in region of 120 kg/mol to 378 kg/mol. This assumption can be valid for simplification purposes, based on the understanding of $T_{g,\text{bulk}}$ of a linear polymer. $T_{g,\text{bulk}}$ of polymer is independent of molecular weight once its MW passes a segment molecular mass, and the segment molar mass of PS is between 30 kg/mol and 40 g/mol as determined by the thermo-mechanical method (Askadskiĭ 2003). This is further confirmed in work of Zhang et al, where bulk PS is prepared by annealing PS nanoparticles and the $T_{g,\text{bulk}}$ of PS measured has a similar value as reported (Zhang, Guo et al. 2011). In my study, the PS nanoparticles in final dispersion with MW of 48,100 g/mol or 75,700 g/mol (larger than segment molar mass) are believed to share similar Tg behavior as the PS nanoparticles in work of Zhang et al, with particle radius around 100 nm (diameter of 200 nm). They have $T_{g,\text{surf}}$ larger than RT so the PS polymer chains are still in glassy state even when considering the Tg confinement effect. On the other hand, the Tg confinement effect of PMMA is more complicated than PS based on PMMA thin film study (Zhang, Guo et al. 2011). Although no report has been found on Tg of PMMA nanoparticles in aqueous solution, hydrogen-bond formation between carbonyl oxygen (-C=O) of PMMA and water molecules has been reported. Water molecules can penetrate into PMMA thin films, form a swollen layer and lead to a slight increase of film thickness after water immersion (Tanaka, Fujii et al. 2008, Lee, Chang et al. 2010). This
helps explain the PMMA behavior in my study. Hydrogen bonds form at the interface of the PMMA nanoparticle and water and the water molecules act like plasticizers which significantly increase the mobility of the PMMA polymer chains by reducing dipole-dipole intermolecular bonding between the chains (Askadskiĭ 2003). Hence, the \( T_{g,\text{surf}} \) of PMMA nanoparticles is likely reduced to a value smaller than RT, so that the PMMA chains at the surface layer are rubbery and leads to film formation and aggregation upon drying as observed.

![Figure 5.15 Tg confinement effect of PS nanoparticles in aqueous solution.](image)

(a) \( T_g \) of PS nanoparticles in solution increases with particle size as measured by modulated DSC (experimental data - solid square, fitting- solid line), \( T_{g,\text{bulk}} \) of PS is around 100°C; (b) Schematic diagram of the gradient interface model of Tg confinement effect of a polymer nanoparticle. Reprinted (adapted) with permission from (Zhang, Guo et al. 2011). Copyright (2011) American Chemical Society.

The flexibility of PMMA polymer chains not only causes aggregation during drying process, but may also be the main reason for the stability and size reproducibility issue with the PSPMMA and PMMA nanodispersions. Before arriving at this hypothesis, some other efforts were tried to address this issue. These efforts included switching from manual injection to auto injection, filtration of the initial solutions, tuning the pH of the aqueous solution with NaOH and a degassing process. Automatic injection, using low concentration NaOH and degassing process does help the situation, but the effect is limited and abnormal size behavior can still occur, as shown in section 5.3.1 and 5.3.2.
Filtering the initial solutions with GF/B 1.6 μm or PTFE 1.0 μm or Nylon 0.2 μm filters does not show a noticeable effect either. The limited effect of these trials further suggests that the reproducibility issue comes from the characteristics of the PMMA polymer chains. The rubbery PMMA shell of both PMMA and PSPMMA nanoparticles enhances aggregation once the particles come in contact. The aggregation can exaggerate the size variations among the trials, which are caused by unavoidable variance of the experimental conditions. There exist variations of experimental conditions for each case even with best effort to keep them the same, such as the magnetic stirring condition by stirring bar, the exact spot of injection, the mixing detail variance during robust mixing process each time, the quality or aging of initial or NaOH aqueous solutions (even if the pH measured stays the same), etc. In work of Aubry et al. and Roger et al. on PMMA nanoprecipitation, the aggregation and repeatability issue is not mentioned and the final PMMA nanoparticles are spherical according to their claims. This may be because they either use PMMA with ionizable groups or introduce surfactants as means to ensure particle stabilization, after ruling out other possible causes. For instance, our system is more controlled compared to the pouring method of Aubry, our PMMA has higher molecular weight, and the concentrations are reasonably within the ouzo region. The ionizable end groups and surfactants not only help stabilize the particles but also have an effect on Tg, since they can influence the interactions between molecules. Hence, introducing stronger interactions between PMMA polymer chains or using stabilizing agents could be a solution to our current problem. The complication due to PMMA in our study proves the importance of nature of materials on outcome of nanoprecipitation. This
issue also hinders the study of the effect of certain factors on blend particle size in this study, such as molecular weight, concentrations and mixing conditions.

The formation of blend nanoparticles by nanoprecipitation is a result of the system minimizing its total potential energy (Sosa, Liu et al. 2016). The interfacial energy of PS-PMMA should be smaller than that of the PS- solvent mixture (THF/water or water) and the PMMA-solvent mixture in order to have blend nanoparticles form instead of pure PS or PMMA nanoparticles. The core-shell morphology may be explained considering the hydrophobicity of the polymers as a way to estimate the interfacial energies. Solubility parameters can be a starting point in estimating hydrophobicity. The solubility of the materials have reported values of \( \delta_{PMMA}(19.6) \), \( \delta_{PS}(18.6) \), \( \delta_{THF}(18.6) \) and \( \delta_{water}(47.9) \) respectively (unit (MPa)\(^{1/2}\)) (Motoyoshi, Tajima et al. 2010). Note these values of solubility parameters may vary slightly depending on the sources. From solubility parameters alone, PMMA is only slightly more hydrophilic than PS. But taking into account the polarity of PMMA and the possibility of hydrogen bond formation between PMMA and water molecules, the affinity between PMMA and water molecules can be stronger than that between PS and water molecules so that the blend nanoparticles have PMMA as the shell. However, there is not enough information to determine whether this morphology is the equilibrium state of the blend nanoparticles or not. Two related observations are: first, the core of blend nanoparticles is not as spherical as the pure PS nanoparticles; second, changing molecular weight of PS from 18,100 g/mol to 75,700 g/mol does not change the morphology of PSPMMA blend nanoparticles. However, no conclusive argument can be derived from these facts.
5.4.2 Hypothesis of Formation Mechanism

Fast mixing is produced in our set up and the mixing level should be macroscopic as for typical stirred vessels. The mixing upon injection of polymer solution to the water reservoir, which is magnetically stirred at the maximum rate available, produces turbulent flow since the flow inside the injection tube is restricted while the water phase is relatively unrestricted (Molpeceres, Guzman et al. 1996). Both the initial polymer concentration and ratio of THF/water are kept low so that the systems are put into a high supersaturation region. As commonly assumed in the literature with similar experimental conditions (section 2.2.1.2), polymer macromolecules collapse upon mixing and a large amount of stable nuclei form initially. In other words, even if the system is in the metastable region instead of the unstable region, the nucleation step is skipped since the collapsed polymer chains are considered larger than the critical nucleation size in the classical notion. For instance, PMMA with MW 14,700 g/mol has $R_{\text{collapsed}}$ around 1.7 nm and PS with MW of 92 kg/mol has $R_{\text{collapsed}}$ around 3.3 nm, as estimated by Roger et al. and Zhang et al. based on mass and density of single polymer chains (Roger, Botet et al. 2013, Zhang, Pansare et al. 2012). Later, the system continues to grow by aggregation among the nuclei instead of molecular addition. The PS and PMMA used in our experiments do not carry known ionizable groups (according to the data sheets from the manufacture). However, surface charges still exists at the hydrophobic surface of the polymeric particles in aqueous solution, which accounts for the stability of some polymer nanodispersions that either do not use surfactants or have ionizable groups (Sosa, Liu et al. 2016, Roger, Cabane 2012). Hence, the aggregation of the growth stage should be hindered coalescence. Initially when particles are all very small, the hindered coalescence
process resembles Brownian coalescence; particles of random sizes have similar frequency of coalescence. Later, aggregation between large and small nanoparticles has much larger probability due to the repulsive forces from surface charge. The repulsive forces in our case should be less strong than the cases of Roger et al. and Zhang et al. with known ionizable groups, which may result in larger size distributions and weaker colloidal stability depending on the details of system. In addition, the effect of the glass transition needs to be taken into account to explain the stability difference among my samples, although the role of glass transition is not widely discussed in the literature on polymer nanoprecipitation. Within the following discussion, I assume that polymer nuclei or nanoparticles with a rubbery outer layer are able to grow by aggregation or Ostwald ripening since the polymer chains at the outer layer are still flexible enough for entanglement. Once the outer layer of a nanoparticle vitrifies, the probability of aggregation or Ostwald ripening of that nanoparticle significantly drops when in contact with another particle or macromolecule.

For control group samples of pure PS and PMMA in this research, the dissolved PS or PMMA macromolecules in initial solutions collapse into stable nuclei upon mixing with aqueous solution. As mentioned above, the $T_{g,\text{surf}}$ of PMMA at the PMMA/water interface should be lower than room temperature (RT), so the PMMA nanoparticles always have a rubbery outer layer and are able to grow by hindered coalescence. On the other hand, in the work of Zhang et al., the $T_{g,\text{surf}}$ of PS at the PS/water interface is 305K (32°C) and this value is larger than RT. In our nanoprecipitation system, the $T_{g,\text{surf}}$ of PS at PS/(THF/water with ratio 1/9) interface should have a slightly smaller value than 305K (32°C) since the presence of THF makes the polymer chains slightly more flexible but
not much due to the low THF ratio. However, there is another effect of nanoprecipitation that needs to be considered, which is the heat of mixing during solvent mixing. A noticeable temperature increase has been observed during mixing of THF and water, so the actual experimental temperature of nanoprecipitation is higher than RT. As a result, the $T_{g, surf}$ of PS at PS/(THF/water) interface may be below the actual temperature during the nanoprecipitation, in which case the PS outer layer would also be rubbery and the PS nuclei could continue to grow by hindered coalescence. Later, when the dispersions cool down to RT, the PMMA and PS samples behave differently. As the temperature of the dispersion decreases, the $T_{g, surf}$ (larger than RT) surpasses the dispersion temperature at certain point and the PS outer shell vitrifies and becomes glassy. The probability of aggregation or Ostwald ripening is significantly reduced when a PS nanoparticle comes into contact with another PS nanoparticle or macromolecule, since they are less likely to entangle together as the polymer chains are more rigid (as shown in Figure 5.16). As a result of the surface charge and glassy state of the PS outer layer, the PS nanodispersion (both original and THF removed) are stable for months or even longer. For PMMA nanodispersions, the $T_{g, surf}$ of PMMA at both PMMA/water interface and PMMA/(THF/water) interface is below RT. At later stages, the frequency of contacts between PMMA nanoparticles is low due to the surface charge, but once the contact happens, aggregation follows since the outer shell of PMMA nanoparticle is still rubbery. Meanwhile, it is harder for the

\[ \text{Figure 5.16 Schematic diagram of polymer nanoparticle aggregation.} \]
aggregated PMMA nanoparticles to rearrange themselves to spherical structure as required by hindered coalescence to narrow polydispersity, especially if the nanoparticles are already large and the core areas are vitrified. If the aggregated nanoparticles cannot return to spherical structures, the reaction limited cluster aggregation process substitutes for the hindered coalescence process and results in large polydispersity and flocculation (Roger, Botet et al. 2013). This may explain why the PMMA nanodispersions are much less stable than PS nanodispersions. It may also explain the reproducibility issue of PMMA nanodispersions: each trial can lead to slightly different initial size distributions, and the differences are augmented by later aggregation processes and sometimes aggregation between large particles happens much earlier in samples, which results in early flocculation. For PSPMMA blend samples, the situation is more complicated due to the additional interaction between PS and PMMA. Initial collapse of macromolecules might result in pure PS and PMMA nuclei and PSPMMA blend nuclei upon THF water interdiffusion. These nuclei aggregate together and grow into blend nanoparticles. Polymer-polymer phase separation happens at the same time before the interior polymer chains vitrify, the more hydrophilic PMMA becomes the outer shell. Once the PMMA shell is thick enough or the core size is sufficiently large, the core area vitrifies and no longer grows. This explains why the core of blend nanoparticles has a size smaller than pure PS nanoparticles in the control groups. However, it is not sure whether the polymer-polymer phase separation completes before the vitrifying happens. Because of the PMMA outer shell, PSPMMA nanodispersions have similar behavior as PMMA nanodispersions. They are prone to flocculation and much less stable than PS nanodispersions.
Both the stability improvement in THF-removed samples compared to the original samples and the observation of sequential injection samples fit into this line of reasoning. Nanoparticles in THF-removed dispersions have smaller hydrodynamic radius due to the solvent partition/swelling effect. Removal of THF makes particles have more compact structures and decreases the mobility of the polymer chains, especially the ones at the outer layer. Both effects reduce the chances of aggregation between large nanoparticles and lead to more stable nanodispersions. In sequential injection samples, the TEM images indicate that the sequential injection leads to formation of mostly pure PS and PMMA nanoparticles rather than PSPMMA blend nanoparticles. For PS-60s-PMMA sample, the PS nanoparticles formed initially in the dispersion do not act as nucleation sites for the PMMA injected later. This may be because the PS nanoparticles are already vitrified and stable, including the PS polymer chains at the particle surface, so they do not entangle with the flexible PMMA chains to form blend nanoparticles. For PMMA-60s-PS sample, small amount of blend nanoparticles are present together with pure PS and PMMA nanoparticles. One possibility is that the blend nanoparticles form during the THF removal process from dissolved PS and PMMA macromolecules. But blend structures have not been observed in PS-60s-PMMA samples, which may undermine this argument. Another possibility is that a small amount of initially formed PMMA nanoparticles that are on the low side of the particle size distribution are still rubbery (for both core and shell) and the PMMA polymer chains flexible enough to aggregate and entangle with later injected PS, and then form into blend nanoparticles.

For the initial formation mechanism, the observations in this study fail to provide additional insights. Both assumptions of homogenous mixing and interfacial turbulence
for initial mixing can lead to blend formation due to the Tg confinement effect and the hindered coalescence growth. Regardless of whether PS and PMMA collapse together and form into blend nuclei or not initially, it is possible that blend nanoparticles form by coalescence of pure rubbery PS and PMMA nuclei before the vitrification of PS, as stated above. It is still an open question whether the PSPMMA blend nanoparticles are in the thermodynamically equilibrium morphology. This is mainly because the blend particle morphology is a result of the interplay of phase separation of the components and the core vitrification, both of which are challenging to measure due to the dynamic and fast nature of the process and the complex interrelation between them. For instance, the timescale of vitrification of the interior polymer chains is affected by particle size growth, real-time local solution conditions, which are determined by the timescales of mixing, interdiffusion of THF and water and interaction between PS and PMMA. The observations of PS(MW)PMMA does not provide much insight either, since the molecular weight affects many aspects at the same time. On one hand, the polymer-polymer phase separation timescale is proportional to number of monomer units by $N^2$; on the other hand, increasing MW can also increase viscosity of polymer solution and slows the interdiffusion of THF and water, hence affect particle growth and the vitrification time of PS (Sosa, Liu et al. 2016, Kiyono, Szikszai et al. 2012). It is difficult to separate these effects just based on the end products in our experiments.

5.4.3 Summary

This work demonstrates the formation of PSPMMA immiscible blend nanoparticles by the nanoprecipitation method in fast mixing and high supersaturation conditions. Aggregation and the formation of a network structure in PMMA are observed. On one
hand, the complication of PMMA in our systems hinders the study of factors such as MW and concentration. On the other hand, it suggests the importance of the nature of polymers especially the glass transition and Tg confinement effect, on the behavior of the final products, including particle size, stability and reproducibility. By applying a model taking into account the Tg confinement effect, the behavior differences of PS, PMMA and PSPMMA blend samples can be qualitatively explained. The finding supplements the current understanding of polymer nanoprecipitation and helps guide future selection of materials to get desired end products. Besides, the film-forming characteristic of PMMA upon drying might be of practical value in study of latex films. In SEM images of dropcast PSPMMA blend samples, the PS nanoparticles are well dispersed in PMMA network. With further treatments and adjustments, latex films with fine domains might be realized with tunable properties.

However, this work does not provide much insight on the early stage of formation mechanism because the limitation of a phenomenological approach of our study and the fast and complicated nature of the nanoprecipitation process under conditions of fast mixing and high supersaturation.
6 EXPERIMENT- NANOPRECIPITATION OF PERYLENE-TCNQ

Nanoprecipitation of perylene and TCNQ co-crystals was based on preliminary work previously done by Nishida et al. in our lab, with similar sample preparation and characterization methods. In this work, the absorption measurement was expanded to mid-infrared region and the powder X ray diffraction was improved to better investigate the composition of nanoprecipitated co-crystal samples. Moreover, the 3 to 1 stoichiometric ratio of perylene and TCNQ were investigated in addition to the 1 to 1 ratio.

6.1 Sample Preparation

6.1.1 Materials

Perylene was purchased from Sigma-Aldrich. 7,7,8,8-Tetracyanoquinodimethane (TCNQ) was purchased from Acros Organics. The good solvent used was acetone from Fisher Science Education. Deionized water was used as non-solvent. HPLC-grade water purchased from Fisher Scientific was also tested as non-solvent. All materials were used directly without further purification.

6.1.2 Nanoprecipitation Process and Sample Information

6.1.2.1 Typical Sample Preparation Procedure

A typical nanoprecipitation procedure employed in this research is described as follows and variations of the sample preparation process performed will be described for the specific sample groups. Before nanoprecipitation, initial acetone solutions of perylene
(“P/A” for short), TCNQ (“T/A” for short) and perylene-TCNQ mixture were prepared. Perylene and TCNQ were dissolved into acetone respectively with molar concentration of 2mM to make P/A and T/A. Perylene-TCNQ initial solutions with two stoichiometric ratios, 1 to 1 and 3 to 1 (P1T1/A and P3T1/A for short), were also prepared. Mixing equal portion of P/A and T/A made P1T1/A; and mixing 3 portion of P/A with 1 portion of T/A made P3T1/A. Unless specified, the initial molecular solutions were freshly prepared on the same day of nanoprecipitation to avoid possible aging effects.

The nanoprecipitation was carried out by manually injecting the initial molecular solution into water at volume ratio of 1 to 9 using a syringe, as shown in the schematic diagram (Figure 6.1). Water was moderately stirred for 1 to 2 minutes. All the experiments were performed at room temperature. Freeze-drying was carried out on 1-hour old samples. The samples were quickly frozen in liquid nitrogen and then placed in the freeze dryer for several days for the water sublimation (in vacuum and at temperature below 0°C). Trials on acetone removal were also carried out on portions of P3T1 and P1T1 nanoprecipitated samples, by constantly stirring the dispersions under the fume hood for certain time.

Figure 6.1 Schematic diagram of perylene-TCNQ nanoprecipitation. Reproduced from (Nishida 2011) with permission.
6.1.2.2 Sample Groups

6.1.2.2.1 P3T1 Sample and Control Group Samples

P3T1 nanoprecipitation was performed by manually injecting 2ml of P3T1 initial solution into 18 ml of water using a syringe. The rate of injection was roughly 0.2 to 0.4 ml/sec. Water was stirred using a magnetic stir bar at 200rpm for about 1 minute. Pure perylene and TCNQ nanoprecipitations were also carried with the same procedure as control trials, directly from P/A and T/A respectively at concentration of 2mM. Two acetone removal trials on P3T1 sample were performed. One trial was done on a 20-minute old sample and the acetone removal lasted for 40 minutes; another trial was carried out on a 1-hour old sample and the acetone removal lasted for 1 hour.

6.1.2.2.2 P1T1 Samples

Unlike the P3T1 sample, it was found that aging the PT/A initial solution and light exposure after nanoprecipitation might affect the measured optical properties measured of the P1T1 sample. The P1T1 sample was usually prepared with freshly mixed PT/A, which means the mixing of P/A and T/A was performed within 5 minutes before nanoprecipitation. The nanoprecipitation process was the same as the P3T1 sample, while the water was stirred at rate of 50 rpm for around 1 minute. The nanoprecipitated sample was left in the normal lab environment with lights on. All the other conditions and procedures were the same as described for the P3T1 sample. Unless specified, the P1T1 notation refers to the “Fresh-Normal” sample just described. P1T1 samples were also prepared under different aging and light exposure conditions. “Fresh-Dark” sample refers to the PT/A that was freshly mixed before nanoprecipitation while the sample was kept in
dark environment after nanoprecipitation. “Aged-Normal” sample refers to when the PT/A was aged overnight or for days in a normal environment before nanoprecipitation and the sample was kept in normal environment after nanoprecipitation. Other trials like “Aged-dark” or aging PT/A in dark cold environment were also tested once. All the nanoprecipitation processes and following procedures were the same as described previously. Acetone removal trials on P1T1 samples were also carried out.

6.1.2.2.3 Unstirred Samples for Early-Time Characterization

P3T1, P1T1 and control group samples were also prepared under unstirred condition, with the notation “No-stir”. The nanoprecipitation process was carried out by injecting 0.3 ml of initial solution into a small glass cuvette containing 2.7 ml of water without stirring. Absorption and DLS measurement were performed immediately after injection. As a result, absorption data could be taken as early as 5s after nanoprecipitation, taking into account manual initiating time, measurement integration time and number of measurements for averaging (integration times of UV-VIS and IR spectrometers were 63 ms and 43 ms respectively, the number of measurements for averaging was 5). Size measurement by DLS was started right after mixing and the first measurement duration was set to 10s. The concentration of P1T1 and P3T1 samples was the same as above, while the concentrations of pure perylene and TCNQ in acetone here were 1mM instead of 2mM.
6.1.2.3 Sample Characterization

6.1.2.3.1 Absorption Spectroscopy

Absorption measurements were performed on nanoprecipitated nano-crystal dispersions. Time dependent measurements were carried out on samples from around 2 to 3 minutes after nanoprecipitation and lasting 40 minutes to 1 hour. Older samples were also measured periodically. For unstirred samples, the measurement started less than 5 seconds after mixing. An acetone water mixture (volume ratio 1/9) was used as a reference solution. The integration times used for UV-VIS and IR spectrometers were set to 63 ms and 43 ms respectively. The number of measurements for averaging was set to 5 for both spectrometers. In an effort to reduce spectrum intensity and to achieve better resolution at lower wavelength (below 500 nm), small portions of original samples were diluted by mixing 1 portion of original sample with 20 to 60 portion of DI water and the measurement was carried out right away. This was a quenching process that did not affect the crystal structure. Diluted reference solutions were also prepared accordingly. The absorption of freeze-dried samples was also checked. The freeze-dried (FD) samples were re-dispersed into pure DI water by sonication. Pure water was used as reference solution. For diluted and FD-redispersed sample measurement, the settings of spectrometers were adjusted to achieve sufficient intensity and resolution. In the following sections, the absorption spectra from UV-VIS and IR spectrometers are plotted on the same graphs. The wavelength region 345 nm to 1010 nm are from spectrum obtained with the UV-VIS spectrometer and the region 1010 nm to 1300 nm are from the IR spectrometer.
6.1.2.3.2 DLS Measurement

Sample growth was monitored with DLS from 3 minutes after nanoprecipitation for a total duration of 40 minutes. The duration of each measurement was set to 30 seconds, with a 10 second wait time between each measurement. Samples that were 1 hour old or older were also measured. For unstirred samples, DLS started within 5s after mixing with a starting duration of 10s and increasing measurement durations later on. The refractive index and viscosity of the medium were set to values of water, with the refractive index equal to 1.33 and viscosity to 0.89 cp. With a ratio of acetone to water ratio of 1 to 9, the change of refractive index and viscosity is minor and considered not to significantly affect the particle size.

6.1.2.3.3 TEM and SEM Sample Preparation

For SEM sample preparation, methods included freeze-drying, spray-drying and drop-casting followed by wicking dry. Freeze-dried samples were either directly put onto the aluminum stub or first re-dispersed into water and then dropped onto the stub and let dry. The spray-drying process was the same as described for the polymer blend sample: 1-hour old crystal dispersions were sprayed carefully several times onto the sample stub and left to dry. Drop-casting was done by dropping a small volume of final dispersion onto the aluminum stub, waiting for 10 minutes and wicking dry the extra solution with filter paper. TEM samples were also prepared by drop-casting and wicking dry after 2 minutes.
6.1.2.3.4 PXRD Sample Preparation

Freeze-dried samples were used for PXRD measurement. A thin layer of grease was applied to the sample holder (round flat plate) and the freeze-dried crystal powders were coated onto the grease. The experiments were run over the weekend under scintillation mode. The scanning rate was set to $0.05^\circ/min$ and the receiving slit was 0.3mm.

6.2 Experimental Results

The experimental results for P1T1 and P3T1 are presented separately in this section due to the complication of P1T1 nanoprecipitation.

6.2.1 P3T1 Nanoprecipitation

![Absorption spectra of P3T1, pure perylene and TCNQ samples](image)

**Figure 6.2 Absorption spectra of P3T1, pure perylene and TCNQ samples.** Spectra of both original and unstirred dispersions at 3 minutes and 1 hour after nanoprecipitation are included. Absorption spectra of acetone removal trial and the FD-redispersed sample are also presented.
Figure 6.3 Absorption spectra of diluted P3T1 samples. The nanodispersions were diluted at 3 minutes and 1 hour after nanoprecipitation. The spectrum of diluted P3T1 FD-redispersed sample is also included.

Figure 6.4 Absorption spectra of diluted pure perylene and TCNQ samples. The nanodispersions were diluted at 1 hour after nanoprecipitation. Spectra of FD-redispersed samples are also included.

Absorption spectra of P3T1, and pure perylene and TCNQ are presented in Figure 6.2 to Figure 6.4. Sample dilution was done right before absorption measurements from corresponding original samples, so the quenching would not affect the crystals in the solution while it just lowered the intensity in the shorter wavelength VIS region to improve resolution. The center peak locations of the major absorption bands of P3T1 match well with the reported P3T1 single crystal from work of Michaud et al. (Michaud,
Carlone et al. 1979). The most intense band reported of P3T1 is located at 452 nm, which agrees with the experimental data here. Another major band located around 420nm is also observed. The reported 445 nm band appears in FD-redispersed sample while it overlaps with the 452 nm band. According to Michaud et al., the bands of P3T1 located between 380 nm to 450 nm are attributed to locally excited perylene. Some additional bands were reported in this region, but they cannot be identified due to noisiness of the signals in the short wavelength VIS region and band overlapping. In the IR region, the bands are very broad and they overlap. Reported P3T1 bands in this region are also very broad but better separated since the spectrum was measured at low temperature (10K and 70K), with rough band centers locating at 720 nm, 840 nm and 960 nm (Michaud, Carlone et al. 1979). Peak fitting is carried out in the IR region using Origin multiple peak fitting function, and a good match is achieved as shown in Figure 6.5. The 720nm and 840 nm bands were assigned to local excited (TCNQ\(^{-}\)) of P3T1 by Michaud. These two peaks also appear in nanoprecipitated TCNQ, which confirms the (TCNQ\(^{-}\)) nature of them. However, these peaks in P3T1 spectrum are not from free TCNQ. According to Michaud et al, perylene-TCNQ (PT) co-crystals carry some similar peaks as pure perylene and TCNQ as a result of the characteristics of their crystal structures (more specifically, the existence of one dimensional perylene TCNQ alternating chains and the chain characteristic) (Michaud, Carlone et al. 1979, Soos, Klein et al. 1975). The 960 nm band of P3T1 is not observed in the perylene or TCNQ spectra. It is considered to be from the charge transfer state, arising from a photon absorption from the ionic configuration \(D^+ A^-\) to the neutral configuration DA (Michaud, Carlone et al. 1979). Overall, the band locations, broadness and relative visual intensity of P3T1 sample agree well with reported
P3T1, and its spectrum is different from pure perylene or TCNQ samples. This observation implies P3T1 co-crystal formation by nanoprecipitation. Moreover, the absorption spectrum of P3T1 shows very good reproducibility among trials prepared under the same experimental condition at different times.

The growth behavior of the absorption spectra of the samples is also shown in the figures. The overall P3T1 bands barely change over the course of 3 minute to 1 hour after nanoprecipitation, except that the background intensity increases in region between 460nm to 760 nm (Figure 6.2 and Figure 6.3) due to increased scattering from nanoparticles. The unstirred P3T1 shows same absorption bands from as early as 5 seconds and the growth behavior is consistent with the stirred sample. Its spectrum has slightly larger intensity, which may be a result of uneven mixing and sample accumulation around the injection point. For pure perylene and TCNQ samples, they show distinct absorption growth behavior different than the P3T1 sample. The overall spectrum of perylene continues to increase over time. The bands of TCNQ increase slightly over time while the background remains the same.

For the FD-redispersed P3T1 sample, the absorption spectrum has the same major bands as the original P3T1 sample, except for the increase of the background intensity in the
VIS region. This indicates that the freeze drying process does not affect the P3T1 nanocrystals. The background increase is also observed in FD-redispersed perylene and TCNQ samples. As a result, the TCNQ peaks of the pure TCNQ sample in the IR region cannot be differentiated from the background signal, which prevents peak comparison with the original TCNQ samples. In addition, acetone removal for less than 1 hour does not change the P3T1 peak locations and only causes a slight increase of peak intensity, which implies that acetone removal does not cause P3T1 crystal structural change. The increase of intensity should be due to the increase of final concentration of particles in the solution after the solvent evaporation process.

![Figure 6.6 Particle growth of P3T1 and perylene.](image)

Particle growth was also monitored with DLS as shown in Figure 6.6. Both regular and unstirred samples of P3T1 show similar particle size growth. The hydrodynamic radius of P3T1 nano co-crystals reaches 70 nm within 30 seconds of nanoprecipitation and slowly grows to around 95 nm in the next 40 minutes. 1-hour old samples were also measured with DLS and the radius reached a value of around 100 nm. The average PDI value of
P3T1 samples is about 0.15, suggesting a moderate polydispersity. For the pure perylene sample, its particle size passes 100 nm within 30s. It is also more polydispersed than P3T1. The nanoprecipitated TCNQ sample shows the same behavior as observed by Nishida, that is, large micro-crystals of TCNQ quickly form after mixing, which impedes the acquisition of reliable DLS data (auto-correlation function) (Nishida 2011). The distinct size and growth behavior among P3T1, perylene and TCNQ may also indicate the P3T1 co-crystal formation. The P3T1 samples (1-hour old original samples) have good size reproducibility with average particle size (hydrodynamic radius) around 100 nm with minor size variation among trials. P3T1 samples are also quite stable: no precipitation is observed for days. Upon short-time acetone removal ($\leq 1\ hour$), P3T1 also shows good
stability and a size reduction compared to the original sample is observed. Note the accuracy of DLS can be limited due to the non-spherical shapes of the crystals in the solution as later observed in SEM images of P3T1 sample. Diffusional behaviors of plate-like particles vary in different directions and polarized DLS is needed to address this issue.

As shown in the SEM images (Figure 6.7), P3T1 nanocrystals have plate-like or parallelepiped-like morphology, which agrees with the cube-like morphology reported on micron-size single crystals of P3T1 by Hu et al. (Hu, Ma et al. 2014). The average size of the P3T1 nano co-crystals in the images is around 200 nm in diameter and the nano co-crystals are moderately polydispersed. This roughly matches with the size information obtained by DLS, although direct comparison is not available due to the limited amount of samples shown in the image and the polydispersity. Perylene samples show similar size and brick-like morphology as reported in nanoprecipitated perylene by Chung et al. (Chung, Kwon et al. 2006). For the TCNQ sample, the image is dominated by the polyhedral structure and a very small amount of amorphous structure is also observed on top of the TCNQ polyhedral plates. The polyhedral structure of TCNQ is also observed in work of Nishida and it is believed to be the thermodynamically stable structure of TCNQ. There are other TCNQ structures observed in nanoprecipitated TCNQ samples of Nishida, however, those structures are not observed here. In all, the P3T1 sample shows different size and morphology than perylene and TCNQ samples in the SEM images, which further implies the formation of P3T1 nano co-crystals by nanoprecipitation. Similar size and morphology of P3T1 nano co-crystals are observed in FD-redispersed samples and spray-dried samples, while the spray-dried sample has a neater look.
PXRD measurements were carried out to study the crystal structures of nanoprecipitated P3T1, perylene and TCNQ samples, which were freeze dried. The experimental PXRD spectra are shown in Figure 6.8 to Figure 6.10, and no corrections were performed on these spectra except the zero shift correction. According to Nishida et al, it is assumed
that the free drying process exerts no impact on the crystal structures of the samples since the cooling rate (by immersion into liquid nitrogen) is much faster than the typical rate of crystal structure change and growth (Nishida 2011, Echlin 2011). This assumption is further confirmed by the consistency of absorption spectra and SEM images between the FD-redispersed sample and the original sample.

The PXRD spectrum of the nanoprecipitated P3T1 sample matches well with the reported spectrum of P3T1 from the Cambridge Structural Database (CSD), in terms of both major peak locations and peak intensities. The reported P3T1 belongs to the triclinic crystal system and P-1 space group (Hu, Ma et al. 2014). The molecular packing of the P3T1 crystal was shown previously in Figure 3.4, with perylene-TCNQ-perylene (P-T-P) alternating stacks and one extra perylene held by weak van der Waals force laterally and perpendicularly in between these stacks (Vermeulen, Zhu et al. 2014, Michaud, Carlone et al. 1979). There also exist a few extra minor peaks (with very small intensity) other than reported P3T1 peaks in the measured P3T1 spectrum at 2-theta locations of 11.4, 13.4, 17.5, 23.5, 25.4 and 29.2 degrees. These peak locations match well with the major peaks of perylene, which indicates that very small amount of pure perylene nanocrystals are present in the P3T1 sample. Meanwhile, no peaks of pure TCNQ nanocrystals are observed in the P3T1 spectrum. The existence of free pure perylene in P3T1 sample may be due to experimental errors occurred from initial material weight variation or initial P/A and T/A volume measurement variation, which leads to a slightly excess amount of perylene. It is also possible that very small amounts of both free perylene and TCNQ crystals form in the P3T1 sample, but that the TCNQ signal is not detected for some reason. In the PXRD spectrum of nanoprecipitated perylene, both peak locations and
relative peak intensities agree well with reported perylene with alpha polymorph. Chung et.al also got alpha form perylene with similar crystal structure by the nanoprecipitation method (Chung, Kwon et al. 2006). For the TCNQ sample, its peak locations match well with reported values but the peak intensities have large differences. The peak intensity discrepancy may be due to preferred orientation during PXRD measurement, since the PXRD sample holder used is a plate holder while the micro-crystals of TCNQ have thin, flat polyhedral morphology. Overall, the PXRD spectrum of P3T1 directly confirms the P3T1 formation by nanoprecipitation method and the P3T1 nano co-crystal dominance in the sample.

6.2.2 P1T1 Nanoprecipitation

6.2.2.1 P1T1 Fresh-Normal Samples

![Figure 6.11 Absorption spectra of P1T1 samples. It includes spectra of 1-hour old P1T1 sample and FD-redispersed sample, as well as spectra of 1-hour old P3T1 and TCNQ dispersions.](image)
Figure 6.12 Time resolved absorption spectra of P1T1 sample. Nanodisperions at 3 minutes, 30 minutes and 1 hour after nanoprecipitation were measured.

Figure 6.13 Absorption spectra of diluted P1T1 samples. It contains 10-min old and 1-h old P1T1 samples (diluted); 1-h old P3T1 sample (diluted); and FD-redispersed P1T1 and P3T1 samples (diluted).

Figure 6.14 P1T1 peak fitting with Origin in IR region. Spectrum of 1-hour old P1T1 sample is used. Peak fitting setting: Gaussian peak type, initial peak number 8; major peak locations: dark purple-overall fitting, red-1038 nm, green – 969nm, blue- 911nm, light blue-835 nm, yellow-763 nm, two minor peaks located at 744 nm and also 835 nm, and a very broad background peak.
Absorption spectra of P1T1 are presented in Figure 6.11 to Figure 6.15. Sample dilution only causes band intensity reduction in order to achieve better resolution at lower wavelengths in the VIS region, as previously described in the P3T1 section. As shown in Figure 6.13, the spectrum of the P1T1 sample in the region between 390 nm and 550 nm has a broad band with strongest intensity around 390 nm and a nearby smaller band around 450 nm. In work of Michaud et al, P1T1 single co-crystals have broad and strongest bands located at around 450 nm, 480 nm and 520 nm instead, a small band near 395 nm, and a medium band at 420 nm (Michaud, Carlone et al. 1979). The discrepancies in band intensities between the P1T1 sample and that reported may be explained by the presence of pure TCNQ or P3T1 crystals or both in the P1T1 sample. Both absorption spectra of TCNQ and P3T1 have strong broad bands centered near 390 nm (Figure 6.4 and Figure 6.13), and their contribution could help justify the unusually high intensity of the 390 nm band observed. In addition, the 450 nm, 480 nm and 520 nm bands are the three strongest bands of reported P1T1, however, the 480 nm and 520 bands of our P1T1 sample have low intensity and are covered in the broad 450 nm band which shows relatively strong intensity. This suggests that the amount of P1T1 nano-crystal in the sample is relatively small and the presence of P3T1 (which has the highest peak at 450 nm) helps drive up the 450 nm band. The bands in this low VIS region are attributed to locally excited TCNQ and perylene derivative according to Michaud et al. (Michaud, Carlone et al. 1979). In the IR region, the P1T1 sample has broad and continuous bands located at around 730 nm, 750 nm, 830 nm, 906 nm and 1030 nm (Figure 6.11). The 730 nm and 750 nm bands are not reported for P1T1 in the work of Michaud but they exist in nanoprecipitated TCNQ sample, and it further supports the presence of pure TCNQ
crystals in the P1T1 samples as suggested previously. Both the reported P1T1 and our TCNQ samples have the 830 nm band, which corresponds to locally excited (TCNQ\textsuperscript{−}) (Michaud, Carlone et al. 1979) The reported P1T1 also has two charge transfer bands centered at around 940nm and 1040 nm, which are shifted slightly from the observed 906 nm and 1030 nm band locations of P1T1 sample. Note that the absorption bands of both the P1T1 sample and the reported P1T1 are broad, so that the values of the peak locations are not precise. There is also the possibility that the peak intensity difference and peak shifts are caused by other reasons such as the difference in experimental conditions and treatment techniques, etc. Peak fitting with Origin (using a multiple peak fitting function) in the IR region was performed based on the assumption that the P1T1 sample is a mixture of crystals and the IR region spectrum consists of peaks from P1T1, TCNQ and P3T1. A good match is achieved as shown in Figure 6.14. This implies that the hypothesis that the sample is a mixture is one possible explanation of the absorption behavior. This matching result is not unique due to the broadness and continuous/overlapping nature of the absorption spectrum. Numerous matching fitting results can be achieved by changing initial values of number of peaks, peak location and peak type, etc.

The growth behavior of the absorption spectrum of the P1T1 sample was also monitored. Figure 6.13 shows that the bands in the region from 350nm to 470 nm do not show obvious change from 10 minutes to 1 hour after mixing, although the signal is too noisy to get detailed information. In the IR region in the time from 3 minutes to 1 hour (Figure 6.12), the 730nm, 750nm and 830nm bands grow slightly over time, which is consistent with the pure TCNQ band growth behavior as shown in Figure 6.2. The 906 nm charge
transfer band does not show any obvious change. The 1030 nm charge transfer band grows from 3 minutes to 20 minutes but then barely changes from 20 minutes to 1 hour. The increase of the 1030 nm peak may indicate more P1T1 co-crystal is forming from 3 minutes to 20 minutes. The absorption spectra of the unstirred sample from 10 seconds to 1 hour are also measured and the same behavior as in the regular sample is observed, although the unstirred sample has a stronger 1030 nm peak.

For the FD-redispersed P1T1 sample, the relative intensity of the bands in the lower VIS region significantly changes from the original sample. The relative intensity of the broad 390 nm band is largely reduced and becomes comparable to the 450 nm band. As a result, the bands in this region show more obvious band characteristics of P3T1 bands, see Figure 6.13. From Figure 6.11, it can be seen that the background intensity in the VIS region of the FD-redispersed P1T1 sample increases; the same behavior is observed in all FD-redispersed samples of P3T1, perylene and TCNQ. In the IR region, the 730 nm, 750 nm and 830 nm bands of P1T1 sample disappear, which agrees with the band behavior of the FD-redispersed TCNQ sample. This further confirms the existence of free TCNQ in the original P1T1 sample. In addition, the relative intensity of the 906 nm band becomes smaller than that of the 1030 nm band.

P1T1 samples were also prepared with slightly different conditions. In Figure 6.15, the 1030 nm band of the unstirred sample has larger intensity, which may be caused by the uneven mixing/sample accumulation visually observed after injection. When HPLC water used as non-solvent instead of DI water, the absorption spectrum only varied slightly. In addition, the absorption spectra of nanoprecipitated P1T1 samples (1-hour old) show good reproducibility among multiple trials with similar experimental conditions.
Based on the absorption spectra behaviors analyzed above, it is highly likely that the nanoprecipitated P1T1 sample consists of P1T1, TCNQ, and P3T1 rather than pure P1T1. However, this finding cannot be concluded just based on absorption spectra because of the broad nature of the bands. More evidence is needed from other characterization methods to achieve a more comprehensive understanding of the composition of the P1T1 nanoprecipitated sample.

The growth of P1T1 particles was monitored with DLS as shown in Figure 6.16. Although uneven mixing and sample accumulation is observed in the unstirred sample,
both regular and unstirred samples have similar particle size growth behavior. The hydrodynamic radius of the P1T1 sample reaches 100 nm within 30 seconds of nanoprecipitation and slowly grows to around 150 nm in the next 40 minutes. The average PDI value is about 0.5, which suggests that the P1T1 sample is highly polydispersed. Measurement of 1-hour old samples from different trials was also performed to test the size reproducibility. Sizes varied from around 100 nm to 170 nm among trials and all samples had large DI value. Similar as discussed in the P3T1 section, the accuracy of DLS is limited when particles are not spherical (the needle and plate shaped crystals are shown later in SEM and TEM images).

SEM and TEM images of the P1T1 samples are shown in Figure 6.17. The P1T1 samples consist of particles with different morphologies, including parallelepips-like (or plate-like) particles and rod or needle-like particles. The spray dried sample shows clearer structures of both plate-like and needle-like particles than the FD samples. The freeze-drying process causes crystals stick together and makes the samples hard to fully re-disperse back into water. There still exist chunks of crystal aggregates after sonication and the re-dispersion process may cause micron-long rod-like P1T1 co-crystal to break into shorter ones. These images also show large size distribution of the particles. This observation agrees with the DLS findings. One possible explanation of the images is that the P1T1 nano co-crystals contain more than one possible morphology. Another explanation is that the different morphologies come from different crystals. For reported microcrystals of P1T1, P1T1 has a needle-like morphology (Hu, Ma et al. 2014). Hence, it is likely the needle-like crystals in the sample are P1T1 crystals. The plate-like structures in the sample could be P3T1 crystals since they resemble the structures of
P3T1 sample shown in Figure 6.7. However, nanoprecipitated TCNQ can have a wide range of morphologies for the same crystal form (Nishida 2011). Overall, just based on the images, it is difficult to come to any definite conclusion concerning the P1T1 sample composition and the assignment of components.

Figure 6.17 SEM and TEM images of P1T1 samples. (a) SEM of P1T1 by FD-redispersed; (b) SEM of P1T1 FD powder; (c) SEM of P1T1 by spray-drying; (d) TEM of P1T1 by drop casting, sat for 2 minutes and wicked-dry.
Figure 6.18 Absorption spectrum and SEM image of 19-hour old P1T1 sample. The SEM sample was prepared with the dropcast-wicked dry method; the sample drop sat on the stub for 15 minutes before being wicked dry. The P1T1 sample can be stable for days without particle precipitation while the particle size increase is observed. Figure 6.18 shows the absorption spectrum and SEM image of a 19-hour old original P1T1 sample. The absorption bands in the IR region barely change from the 1-hour sample while the TCNQ band at 840 nm slightly decreases. The particles in the SEM image show similar morphologies as the 1-hour old sample as well. This suggests that the P1T1 nanoprecipitated samples have pretty good stability. Acetone removal trials on P1T1 samples were also carried out. However, flocculation sometimes occurred upon acetone removal while other times the solutions stayed stable.

PXRD measurements were also performed on P1T1 samples in order to get the crystal structure. Spectra are shown in in Figure 6.19. As noted in the section on P3T1, the experimental PXRD spectrum of the freeze-dried P1T1 sample is only zero-shift corrected and the freeze-drying process is believed to have no impact on crystal structure. Comparing the PXRD spectrum of the P1T1 sample with the reported P1T1 spectrum, it can be seen that they do not match. Instead, the overall spectrum and major peaks (both peak locations and intensities) of the P1T1 sample overlap with the measured P3T1
There also exist additional peaks that belong to P1T1 and TCNQ in the spectrum. The 10.2° major peak (2 theta value) matches with the 10.14° major peak reported in P1T1. The 27.4° medium peak, which partially overlaps with the 27.0° peak, is also present in reported P1T1. In addition, the 10.9° (medium), 18.6° (medium) and 20.1° (very small) peaks of the P1T1 sample overlap with TCNQ peaks. The 13.1° (small but broad) peak exists in both spectra of the P1T1 sample and the nanoprecipitated TCNQ sample, although it is not in the reported TCNQ spectrum. Moreover, no peaks from perylene (alpha form) or P2T1 (reported by Vermeulen et al. (Vermeulen, Zhu et al. 2014)) are observed in the spectrum.

Figure 6.19 PXRD spectra of P1T1 samples and reported P1T1. PXRD spectra of P1T1 at two repeating trials are represented, together with spectra of P3T1 sample.

However, there are issues relating to the quality of the PXRD spectrum of the P1T1 sample. As seen for P1T1 experimental trial 2, the spectrum also resembles the P3T1 spectrum, but unlike the first trial, the major peaks of P1T1 and TCNQ are missing and a strong perylene peak is present. The only P1T1 peak still present is the one at 27.4°. For the P1T1 sample, there has to be free TCNQ or both P1T1 and TCNQ existing in the
sample due to the perylene and TCNQ material conservation, however, their existence is not reflected here by the PXRD spectrum-trial 2. The inconsistency of the PXRD spectra occurs not only in the P1T1 FD samples, but also in filtered P1T1 samples later prepared in our lab. The spectra of P1T1 filtered sample are also dominated by P3T1, which further supports the assumption that the FD process does not affect the crystal structures. The reasons for the PXRD quality issue are still not fully understood, but possible ones include the preferred orientation of P1T1 co-crystal or limited exposure of P1T1 to X ray due to the existence of P3T1 co-crystal, etc. Moreover, although several P1T1 peaks are observed, some major peaks of the reported P1T1 are missing in the spectrum (e.g., the 12.16° and 24.3° peaks). This may be caused by preferred orientation of the P1T1 co-crystal or crystal structure deformation that can lead to uneven peak shifts. Zhu et al. reported a similar issue of XRD spectra of P1T1 nanowires and P3T1 micro blocks prepared by solvent evaporation method of PT mixture in acetonitrile. Only one (120) peak of P1T1 and two peaks (011) and (022) of perylene were observed since the P1T1 nanowire grown along 001 direction (Zhu, Yi et al. 2015). Similar selective peak presentation also occurred for the P3T1 micro block crystals. In all, the quality of PXRD measurement needs to be improved to get a more comprehensive understanding of the composition of P1T1 sample.

To summarize, the PXRD spectrum of the P1T1 sample suggests that its composition is a mixture of P1T1, P3T1, TCNQ and maybe also pure perylene, not only P1T1 co-crystals. The finding matches the expectation from analysis of its absorption spectrum. However, a complete understanding of the composition is hampered by the PXRD quality issue, especially the understanding of the amount of each component in the sample. For
instance, the dominance of P3T1 in PXRD spectrum of P1T1 sample does not explain the relatively strong P1T1 absorption bands in the absorption spectrum.

6.2.2.2 P1T1 Complications

![Figure 6.20 Absorption spectra of P1T1 samples showing complications.](image.png)

**Figure 6.20 Absorption spectra of P1T1 samples showing complications.** The samples were all 1-hour old. Fresh-Normal sample was prepared by fresh P1T1 initial solution and the sample was kept under normal lab environment after nanoprecipitation. Fresh-Dark sample refers to a sample prepared by fresh P1T1 initial solution and the sample was kept in dark after nanoprecipitation. Aged-Normal sample was prepared by aging the P1T1 initial solution (aging time from overnight to days) and the nanoprecipitated sample kept under normal lab environment.

During the tests of the reproducibility of the P1T1 nanoprecipitation sample optical absorption spectra, it was found that aging the initial solution and light exposure after nanoprecipitation can lead to variance of the band intensities. As shown in Figure 6.20, if the P1T1 initial solution (PT/A) is aged overnight or for days before nanoprecipitation, the 1030 nm peak intensity is significantly reduced compared to other samples. The other peaks do not show obvious change. Note the aging of P/A and T/A does not show any effect on absorption bands; the effect only occurs when the mixture of perylene and TCNQ in acetone is aged. We have carried out PXRD measurement on an aged P1T1 sample, and the spectrum is still close to the P3T1 spectrum with additional peaks from
perylen. In work of Zhu et al. on the solvent evaporation method just mentioned, an aging effect of PT in acetonitrile was also observed. There were P3T1 co-crystals precipitated out of the PT/acetonitrile solution (concentration of perylene was between 0.6-0.8 mM while TCNQ was between 1-3 mM) after it was aged for 1 day (Zhu, Yi et al. 2015). According to the author, the perylene and TCNQ molecules of P3T1 have stronger interactions than they do in P1T1 and the P3T1 structure is thermodynamically favorable when the perylene in acetonitrile is larger than 0.6 mM (Zhu, Yi et al. 2015, Truong, Bandrauk 1976). P3T1 also has limited solubility in acetonitrile. It might be a similar case with PT in acetone. The P3T1 is a more stable form than P1T1 in acetone and very small complexes of P3T1 might form during the aging process. As a result, more P3T1 could form in the aged sample of P1T1 nanoprecipitation and it leads to a significant decrease of the P1T1 1030 nm peak. However, the invariance of the 906 nm peaks is not explained. If a freshly nanoprecipitated P1T1 sample is kept in dark, the 1030nm charge transfer band at 1 hour is larger than it is in a normal sample, while the bands in the region of 720nm to 880nm are smaller. The 906nm band stays the same. This indicates more P1T1 formation. Other characterization methods are needed to achieve a better understanding.

6.3 Discussion

6.3.1 Experimental Findings

The experimental setting and conditions were chosen based on previous work of PT co-crystal nanoprecipitation. The initial molecular concentration of PT/A used was equal to or less than 2.5 mM due to the limited solubility of perylene in acetone. The
acetone/water ratio was less than 1:4 in order to achieve PT co-crystal formation. Hence, the initial concentration used was 2 mM and the acetone/water ratio was set to 1/9 in my experiments. When the initial P/T ratio was set to 3/1, P3T1 nano co-crystals formed by nanoprecipitation. According to the PXRD measurement, the P3T1 sample contains mainly P3T1 nano co-crystals and also a very small amount of perylene nano crystals. This is supported by the absorption spectrum of P3T1, which matches well with reported spectra. The P3T1 nano co-crystals had plate-like morphology and their average hydrodynamic radius (1-hour old sample) was about 100 nm. When the initial P/T ratio was 1/1, the nanoprecipitated P1T1 sample was a mixture of P1T1, P3T1, TCNQ crystals and possibly perylene crystals rather than just pure P1T1 co-crystals, as suggested by absorption and PXRD data. The P1T1 sample had a broad size distribution and multiple morphologies were observed in SEM and TEM images. However, the amount of each component in the sample is unknown.

6.3.2 Discussion of Formation Mechanism

As mentioned in the polymer blend section, injecting molecular solutions into a stirred water reservoir produces fast and turbulent mixing. High supersaturation is produced after fast mixing since both perylene and TCNQ have low solubility in the final binary solvent, which has a large fraction of water. For the initial stage of co-crystal nanoprecipitation, the current experimental data fails to provide much insight due to the fast and turbulent nature of nanoprecipitation and the limitation of the characterization techniques applied. For instance, we were unable to tell whether homogeneous mixing can be assumed initially or if interfacial turbulence is a more accurate description, as
claimed by Chuang et al. for perylene nanoprecipitation (Chung, Kwon et al. 2006). The absorption spectra of both P3T1 and P1T1 were measured as early as 5 seconds after nanoprecipitation, and they already have strong charge transfer absorption bands. This observation agrees with the finding in work of Nishida on P1T1 nanoprecipitation, in which the rapid appearance of new absorption bands happened within 1 second after mixing. As reviewed in section 2.2.2, it may be due to fast initial formation of perylene-TCNQ pre-clusters, such as dimers or N-mers, which contribute to the formation of charge-transfer absorption bands within seconds (Nishida 2011, Mori, Miyashita et al. 2009, Van Keuren, Bone et al. 2008). The initial molecular association of pre-clusters happens at a much faster rate than particle size growth (Van Keuren, Bone et al. 2008). However, the size information before 30s is not available in my experiments, so it is impossible to confirm the pre-cluster hypothesis here. The initial mechanism of formation of either pre-cluster or stable nuclei also remains unknown, i.e., whether classical or heterogeneous nucleation or by spinodal decomposition as suggested by Brick et al. (Brick, Palmer et al. 2003). It is also possible that loose bound complex of PT already exist in the PT/A initial solution (Zhu, Yi et al. 2015, Dillon, Bardeen 2012).

In the particle growth stage, the behavior of both P3T1 and P1T1 samples follows a similar pattern of rapid initial increase in particle size that plateaus as the supersaturation is reduced, as observed in other works on small organics nanoprecipitation (Van Keuren, Bone et al. 2008). However, the samples behave differently in the time-resolved absorption measurements. In later growth of P3T1, its major absorption bands do not change over time from 5 seconds to 1 hour after mixing. The average hydrodynamic radius slowly grows from around 70 nm to 100 nm from 30 seconds to 1 hour. The
invariance of the absorption bands upon particle growth indicates that the growth of P3T1 nano co-crystal occurs by a mechanism similar to Ostwald ripening, at expense of either P3T1 pre-clusters or smaller P3T1 nuclei that form in the initial stage. Larger stable P3T1 nuclei or co-crystal continues to grow by collecting molecules diffused from disassociated pre-clusters or nuclei. Otherwise, the changes in absorption bands over time (such as the growth of the CT band and the reduction of bands relating to perylene and TCNQ) would be expected if pure perylene or TCNQ molecules or clusters existed in the initial solution. The growth of the P1T1 sample is more complicated. The 1030 nm and TCNQ absorption bands increased in intensity from 5 seconds to 30 minutes while the 906 nm band was independent of time. The average hydrodynamic radius increased from around 100 nm at 30 seconds to about 150 nm at 30 minutes. Later, from 30 minutes to 1 hour, both absorption bands and size did not show obvious change. The TCNQ bands increased over time and the behavior was the same as observed in pure TCNQ samples, which may indicate more TCNQ crystals forming in both samples. The increase of the 1030 nm CT band with particle growth may indicate formation of more P1T1 co-crystal, however it conflicts with the invariance of the 906 nm CT band over time. There is not yet enough information to fully explain the observations or to understand the particle growth mechanism.

Both P3T1 and P1T1 samples showed good stability after nanoprecipitation, and could maintain stability for hours or days without bulk precipitation being observed. Slight increases in particle size were observed over time and could be due to Ostwald ripening. Since no stabilization agents were introduced into the systems, the stabilization mechanism may relate to electrostatic repulsion. Zeta potential measurements of both
P3T1 and P1T1 samples indicated positive zeta potential, which supports the electrostatic stabilization mechanism.

6.3.3 Summary

In conclusion, this work demonstrates the formation of P3T1 nano co-crystals by nanoprecipitation if the initial perylene/TCNQ ratio was 3/1. For initial perylene/TCNQ ratio of 1/1, the resulting sample was not just P1T1 nano-crystals as expected but is composed of P3T1, TCNQ and possibly perylene. This shows that the final product of co-crystal nanoprecipitation does not just depend on the initial stoichiometry of the components. A detailed understanding of why the P1T1 sample is a mixture and the corresponding formation mechanism could not be determined based on current experimental results. Thermodynamically, the final products should be a result of the system minimizing its energy. However, it is challenging to determine the interactions among different materials during nanoprecipitation. This is the main reason for the current lack of systematic study on the crystal growth of binary charge transfer compounds, especially ones with multiple possible stoichiometries (Hu, Ma et al. 2014). In the nanoprecipitation method, the kinetics may make the situation even more complicated. This work also finds that aging of initial PT molecular solution and light exposure after nanoprecipitation cause intensity changes of absorption spectra of P1T1 sample.
7 FUTURE RESEARCH

The results of this work on polymer blend nanoprecipitation suggest various avenues for future research. One possible area to explore is application related. As shown in the SEM images, the dropcast sample of PSPMMA results in a PMMA film with fine domains of PMMA, as a result of the film formation characteristic of the PMMA part. Keddie et al. reported ways of producing latex films from colloidal polymer nanocomposites (Wang, Keddie 2009). Our PSPMMA sample may be a good candidate for forming good quality latex films if a proper casting method can be found. The mechanical properties of the PSPMMA sample can be measured and the effect of annealing can also be tested. Other ingredients in addition to PSPMMA can also be added to the nanoprecipitation system to tune the properties. For instance, compatibilizers or copolymers can be used to improve the mechanical properties and film stability. There are also many questions remaining unaddressed in understanding the results of the current work. First, the composition of the core of the blend nanoparticle need to be investigated to see whether it is mostly PS or well entangled PS and PMMA. Besides, the stabilization mechanism of the PSPMMA system is not clear, but could be investigated by, for example, zeta potential measurements. Some preliminary trials on a PSPMMA sample have shown negative zeta potential, but these would need to be re-confirmed with a careful systematic experimental design, similar to the work of Roger et al. and Zhang et al. (Roger, Botet et al. 2013, Zhang, Pansare et al. 2012). Efforts also need to be made to control particle aggregation and improve sample stability by using PMMA with known ionizable groups and with proper pH and ionic strength control (if surfactantless colloids are desired). In work of Roger et al. the PMMA used has molecular weight of 14,700 g/mol and carboxylic acid
end groups and good particle stability was achieved when the pH and ionic strength are set to desired values (Roger, Botet et al. 2013). Similar PMMA could be used with acetone as good solvent, since the same PMMA is also used in work of Aubry et al. and their PMMA/Acetone/water systems are well studied and their understandings can be directly borrowed, including the phase diagram and growth behavior. Once the reproducibility and aggregation issues are addressed, factors such as the PS/PMMA ratio, the polymer molecular weights, concentrations, solvent/non-solvent ratio, and temperature at which the nanoprecipitation is carried out could be further studied.

Another interesting line of research would be to study confinement effects on the glass transition temperature. The polymer blend nanoprecipitation method can also be expanded to other polymers. The characteristics of the polymers used, such as the glass transition temperature and the solubility in various solvents can be used to help choose the polymers. For instance, if a polymer has low \( T_{g,\text{bulk}} \) or is able to form hydrogen bonds with water (containing groups such as \(-\text{OH}, -\text{C} \equiv \text{N}, -\text{C}=\text{O} (-\text{O})\)), the resulting nanoprecipitated polymeric particle may have similar aggregation problems or film formation characteristics upon drying as observed in this research. Otherwise, if a polymer has similar or larger \( T_{g,\text{bulk}} \) than PS and has property similar to PS, the resulting particles maybe very stable.

For co-nanoprecipitation of perylene and TCNQ, more work needs to be done to better understand the system. For the P1T1 sample, trials can be carried out to form pure P1T1 nano-cocrystals instead of a mixture of different crystals. Changing the solvent/non-solvent combination is a promising strategy for this. In the work by Hu et al. using solution crystallization methods for forming PT single co-crystals, a selective
crystallization effect was observed, and whether P1T1 or P3T1 co-crystals formed depended on the solvent used (Hu, Ma et al. 2014). This suggests that the solubility plays an important role in the final products. In our system, using a solvent other than acetone or a different non-solvent might help control the final outcome and achieve only P1T1. However, finding the right solvent and non-solvent combination is not always easy and an ideal combination might not exist. Various other factors in PT nanoprecipitation can also be investigated, including the P/T ratio, the initial concentrations, the solvent/non-solvent ratios and the temperature. We have carried out preliminary studies of some of these, but more work is needed to confirm the trends observed. For instance, when using P/T ratio of 1/1.5 and 1/2, the 1030 nm band increased significantly, indicating formation of more P1T1. However, those samples are more polydispersed and less stable, possibly due to the presence of excess TCNQ. Nishida also tested the temperature dependence of P1T1 and observed significant peak shifts. The mixing condition must be better controlled and monitored by applying automatic nanoprecipitation geometry to achieve a systematic study of the factors. For potential application, nano co-crystal samples of higher quality are required and the electronic and photovoltaic properties of the co-crystals need to be investigated (Hu, Ma et al. 2014). The co-crystal nanoprecipitation can also be expanding to other material combinations, such as tetrathiafulvalene (TTF), naphthacene (Tetracene) as electron donors and Tetracyanoethylene (TCNE) and TCNQ as electron acceptors. Some of the combinations are being tested in our lab.
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