DYNAMICS OF DENSE MICROGEL SUSPENSIONS

A Dissertation

Submitted to the Graduate School
of the University of Notre Dame
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

by

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December 2014
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Abstract

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Microgels are a multifaceted material with a broad range of applications including biomimicry and drug delivery. Aqueous microgels are micron-sized hydrogel particles composed of a swollen network of interconnected polymers. Based on the properties of the polymers, microgels can be designed to be stimuli-responsive or to act as model colloidal particles. Chapter 1 details the background and uses of microgel particles. As dense suspensions of microgels tend to form colloidal glasses at high concentrations, the opening chapter provides a strong focus on the glass transition and details certain aspects including glass fragility, dynamic heterogeneity, and the confinement effects.

Starting in Chapter 2, common methods of microgel synthesis are introduced, with a focus on emulsion polymerization. This is the method used throughout this dissertation to synthesize microgels based on two polymer backbones: poly(N-isopropylacrylamide) (PNIPAM) and poly([2-(Methacloyloxy)ethyl]-dimethyl-(3-sulfopropyl)] ammonium hydroxide) (PMSA). PNIPAM has a lower critical solution temperature (LCST) of 32°C, which has led to significant interest for biomedical applications. PMSA is a zwitterionic
polymer derived from molecules found in the cell membrane, making it highly biocompatible. In Chapter 3, these microgels are employed to replicate the properties of lubricin, a globule protein, with the goal to develop a biomimetic super-lubricant inspired by the synovial fluids. It is demonstrated that the unique rheological properties and low friction behavior is replicated through mixtures of microgels and hyaluronic acid, a biopolymer also found in the synovial fluids.

The remainder of this dissertation discusses a direct microscopic study of the glassy dynamics of microgel suspensions using particle-tracking algorithms. In Chapter 4, it is demonstrated that tuning the elasticity of microgel particles changes the glass fragility from fragile to strong glass-forming behavior. In Chapter 5, these glassy suspensions are shown to be spatially heterogeneous and links are developed between glass fragility and dynamic heterogeneity. The growth of dynamic heterogeneity is found to be directly related to the glass fragility. In Chapter 6, deviations in particle dynamics are examined for strong glass-forming suspensions under strong spatial confinement. Interestingly, the confinement length scale is shown to be less than the length scale for hard-sphere suspensions.
This is for all of my family, friends, & teachers
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**Figure 6.5:** The normalized four-point correlation function, \( g_4^*(\tau,r) \), at varied lag times, \( \tau \), for \( E^* = 169 \text{Pa} \) & \( \phi = 0.7 \) for confinement heights: (a) \( h/d_H = 52 \), (b) \( h/d_H = 22 \), (c) \( h/d_H = 16 \), & (d) \( h/d_H = 14 \). Colors correspond to lag times, \( \tau = 75.1 \text{s} \) (black), \( \tau = 751 \text{s} \) (red), \( \tau = 1.88 \times 10^3 \text{s} \) (green), \( \tau = 7.59 \times 10^3 \text{s} \) (blue), & \( \tau = 1.32 \times 10^4 \text{s} \) (cyan). .......................................................... 105

**Figure 6.6:** (a) The dynamic length scale, \( \xi_4 \), as a function of confinement height, \( H/d_H \). (b) Same as (a) with \( \xi_4 \) scaled by the separation distance, \( R_0 \). ............................................. 107
ACKNOWLEDGMENTS

Above all, I would like to thank my research advisor and mentor, Dr. Yingxi Elaine Zhu. None of this would be possible without her constant advice, support, and funding. I believe her passion to understand the fundamentals of science improved me as a scientist and a researcher. Her advising style developed me as a better writer, speaker, and teacher, and she gave me the freedom to explore international and industry research settings in order to fine-tune my career goals. It was a privilege and an honor to work in her research group.

Secondly, I would like to thank all of the professors who have provided me guidance through the years. First, I would like to thank Dr. Noelle Comolli from Villanova University for introducing me to the fields of soft matter and polymer science while I was an undergraduate student. I would also like to thank Dr. Zexin Zhang from Soochow University for hosting my stay in China and for the many fruitful discussions regarding particle tracking and glassy dynamics analysis. I want to thank Dr. Arezoo Ardekani, now at Purdue University, for advice and guidance in rheology and complex fluidics. Lastly, I would like to thank my thesis committee members, Dr. David Leighton, Dr. Davide Hill, and Dr. Jonathan Whitmer, for taking time out of their busy schedules to serve as my thesis readers.
I am also grateful of the past and present group members who have helped me along the way. I especially would like to thank Dr. Prasad Sarangapani for laying the groundwork on the particle tracking analysis and the glassy dynamics project, and the many fruitful discussions and guidance he gave me along the way. I also want to thank Dr. Benxin Jing for his help and expertise with many experimental methods and instrumentation. I would like to thank the undergraduate students and visiting researchers who participated in my research: Rachel Dever, Kerry McNeilly, and Wenshuai Dong. I would like to mention all the group members who I have worked with throughout the years, in no particular order: Dr. Victoria Goodrich, Dr. Zhongli Zheng, Collin Gurtowski, Na Qi, Chen Qu, and Brian Yoo.

Finally yet importantly, I want to thank my family and friends who have supported me through graduate school. My family has always promoted my efforts to achieve higher education and my graduate experience would have been much less enjoyable if it was not for the friendships I established here at Notre Dame. I especially would like to thank Patricia Rafferty for her constant encouragement to pursue my dream career in research and science and her help to make that dream a reality.
CHAPTER 1:
AN INTRODUCTION TO MICROGELS & MICROGEL SUSPENSIONS

1.1 Overview of Hydrogels & Microgels

Synthetic hydrogels are a highly versatile polymeric material with a wide range of dynamic properties. [1-3] Their applications range from culinary arts to catalysis to biomedical sciences. [3-12] The focus of this dissertation is colloidal hydrogel particles, or microgel particles. With sizes ranging from nanometers to micrometers and with strongly tunable properties, microgels can mimic many components of biology as well as act as model colloids. [13-15] Here, in the first part of this chapter, the structural makeup and past applications of hydrogels and microgels will be reviewed. The second part of this chapter will focus on glassy dynamics, specifically how it relates to dense microgel suspensions. This chapter will conclude with a brief outline of the rest of the dissertation.

1.1.1 Introduction to Hydrogels

Hydrogels are found frequently in nature, make up a larger percentage of many biological constituents, and are commonly experienced and used on an everyday basis. By the simplest definition, hydrogels are swollen aqueous networks of linked polymers. [1-3] Many gelatinous foods, such as Jell-O, are hydrogel structures where the polymer
backbone is composed of gelatin and sugar molecules. [16] In human anatomy, many components of the human body are hydrogels, e.g. the articular cartilage, the stomach wall, and the lens of the eye, formed by crosslinking proteins, fats, and other biopolymers. [3] The tertiary structure of proteins, for example, can be considered a hydrogel composed of biopolymers, e.g. proteins, which are strongly hydrophilic. [17-18]

A study by Wichterle and Lim in 1960 demonstrated the use of polymeric hydrogels for contact lenses and since then hydrogels have been target of interest for many biological applications. [3,19] These desirable qualities have led to a range of medically-inspired applications using hydrogels, including cosmetics and wound treatment. [3-6] They are a pliable material with very similar properties to natural tissue, which has led to significant interest for tissue regeneration. Hydrogels are also highly porous, leading to research focused in its transport properties for the design of catalyst supports and drug transport. [7-10]

The network formation of hydrogels can be either reversibly or irreversibly crosslinked together. [1-3,20] Molecular entanglements and secondary forces, i.e. ionic, hydrogen bonding, and/or hydrophobic forces, between polymer strands in solution, form a reversible hydrogel, or “physical” hydrogel. [20] These types of binding forces lead to the high heterogeneity and low stability typically found in physical hydrogels. Environmental conditions can strongly alter the strength of the secondary forces which could lead to faults in the mechanical stability of the hydrogel. An irreversible hydrogel is formed by chemically crosslinked polymer strands, usually facilitated by a crosslinking agent, which is typically a molecule with a similar structure to the monomer of the
polymer. [20] These types of hydrogels are often more homogeneous and are influenced less by environmental stimuli.

Due to a high degree of porosity, a hydrogel can contain up to 99.9% water, which amounts to thousands of times of the weight of the polymer backbone. The high water content in the hydrogel is due to hydrophilic groups, e.g. amino, carboxyl, or hydrogel groups, on the polymer chain. The total water content inside the hydrogel is a tunable variable dependent on synthesis parameters, e.g. the degree of crosslinking and the number of hydrophilic groups, and on environment stimuli, e.g. temperature and pH. Increasing the crosslinking density will reduce the free volume inside the hydrogel and create a more rigid structure. This will limit the total water content able to absorb into the material, which prevents it from swelling. A higher degree of hydrophilic groups will increase the water content inside the hydrogel. Depending on which hydrophilic groups are present in the polymer, the degree of hydrophilic groups could increase or decrease by changing the temperature or the pH of the aqueous solution.

1.1.2 Microgels: Micron-sized Hydrogel Particles

One subset of hydrogels that has gained significant interest of late is colloidal sized hydrogels particles, or microgel particles, in aqueous solution. [13-15] They are in a class of macromolecules between branched polymers and macroscopic polymer networks. The size of a microgel particle can be as small as several nanometers or as large as several micrometers. This is strongly dependent on the molecular weight of the polymers, degree of crosslinking and the nature of the solvent. [21] The size of the particle is inversely related to the crosslinking density, as the polymers pack more tightly in the network
when there are more linkages, which reduces the degree of swelling. Similarly, the degree of hydrophobicity will also govern the swelling behavior of the particle and therefore control the size of the particles as they will swell in good solvent conditions and collapse in poor solvent conditions. Additionally, the crosslinking density and the degree of hydrophobicity also control the elasticity of the particles. [22-23] Swollen particles will have a high solvent quality that will lead to higher mechanical compliance, where collapsed particles will be more rigid.

These particles have the ability to act as model colloidal particles by tuning several readily controlled parameters. [13-15] Microgel particles with a high degree of crosslinking tend to behave as hard-sphere colloids. Decreasing the crosslinking density leads to deformable microgels that are strongly influenced by their environment. The size of deformable microgels is dependent on the osmotic pressure of the sample. Increasing the concentrations of microgels or adding solute molecules to the solution will decrease the size of the microgel. Furthermore, changing the solubility of the microgel will control its size. In a good solvent, a microgel will swell but in a bad solvent, it will collapse. More interestingly, the elasticity of the microgel is also controlled through the solution properties. Swollen microgels in a good solvent will have a lower elasticity than collapsed microgels in a poor solvent.

1.2 Glassy Behavior of Microgel Suspensions

Dense suspensions of microgels will form a disordered state rather than crystallize at high concentrations, resulting in a glassy material. [23,25-28] As the glass transition is approached, the particles will enter a jammed state, and the mobility of the microgels will
decrease greatly. This leads to an increase in the dynamic properties of the sample, e.g. the viscosity and relaxation time. Mattsson et al showed this in a study for microgels of variable elasticity. [23] The concentration dependence of the relaxation time is reproduced here in Figure 1.1. The rate at which the relaxation time increases is highly dependent on the elasticity of the particle. Hard-sphere particles form fragile glasses due to a super-Arrhenius increase in viscosity. [28] These particles will remain mobile in solution until the particles in the suspension become jammed and the motion of particles becomes arrested. Alternatively, if the particle is deformable, it will exhibit strong glass-forming behavior characterized by Arrhenius increase in viscosity. [29] However, the cause for this behavior is not well understood or agreed upon at present. In this section,

Figure 1.1: (a) Plot of the scaled relaxation time, $k\tau_0$, versus effective volume fraction, $\zeta$, for stiff (diamonds), intermediate (circles & squares), and soft (triangles & crosses) microgels. (b) Same as (a) where $\zeta$ is normalized by the glass transition volume fraction, $\zeta_g$. Reprinted from ref. [23].
the glass transition will be briefly reviewed. It will explain why previous studies employed colloidal suspensions as model glassy systems.

1.2.1 Background of the Glass Transition

To examine the glassy dynamics of microgel suspensions, it is important to understand the fundamentals of the glass transition. Glasses are often found in everyday life and in many industrial processes. A molecular glass is a disordered material that lacks the structured order characteristic of crystalline materials. [25] Typical molecular glasses include silica oxide, which forms the glass used in windows and beakers, and poly(methyl methacrylate), which is used to make Plexiglas. Initial studies in glassy dynamics investigated thermal glasses, or molecular glasses, and showed that the glass transition is induced by rapidly quenching the temperature of the liquid. [25-26]

Generally, a molecular glass forms when a liquid is quenched at a fast cooling rate and the molecules fail to form crystalline order. [25] While the liquid to crystalline solid phase transition is an abrupt, first order transition, the glass transition is a continuous process characterized by a gradual increase in dynamic properties, e.g. viscosity and relaxation times, as the material transitions from liquid-like to solid-like behavior. [25,27] This transition does not occur at a fixed temperature, rather, it depends on the rate of heating and cooling. This route to the glassy state is important to many industries that involve the formation of glasses, yet there is much about it that is not yet fully understood.

As the quenching rate plays an important role in glass formation, the glass transition is viewed as a three phase jamming process which typically which involves both
temperature and time. [25-28] This means that particle dynamics are an intricate part of the glass transition. The three phases include a super-cooled phase separating the liquid and glass phases. At high temperatures, the material exists as a viscous liquid. [25,30] When the temperature is lowered at a fast enough rate to avoid crystallization, the material enters the super-cooled phase, which is characterized by a sharp increase in the viscosity. [29-31] Further decreasing the temperature will continue to increase the viscosity, and eventually the material will deviate from liquid-like behavior to solid-like behavior as it transitions from the super-cooled phase to the glassy phase. As the super-cooled phase can exhibit aspects of a liquid phase and a solid phase, understanding the phenomena associated with this phase is essential to many industrial processes involving glasses.

While molecular glasses are more commonly known, colloidal suspensions, emulsions, and granular materials also form disordered glassy materials. [25] Typically, for these types of materials, the dynamic properties are dependent on the concentration of particles or the discontinuous phase rather than the temperature. These systems, especially colloidal suspensions, have been the interest of many experimental studies hoping to probe the glass transition at the particle level. While the particles in a molecular glass are too small to be seen using dynamic microscopy techniques, colloidal particles, with sizes on the order of a micron, are prime candidates for microscopy studies. [27] Also, their movements are much slower than individual molecules, which can be captured via traditional microscope video cameras.

Many previous studies have focused on colloidal suspensions to elucidate the phenomena in molecular dynamics. Colloids can be designed with a wide range of
tunable properties to mimic the behavior of molecules under investigation. Idealized model colloids act as hard-spheres in solution and are impenetrable with no attractive or repulsive interactions except for excluded volume interactions. Such colloids are described by the following potential, $U(r)$, equation. [27]

$$U(r \leq 2a) = \infty, U(r > 2a) = 0$$  \hspace{1cm} (1.1)

Here, $a$ is the radius of the colloid. As described by this equation, the particles will have an infinite repulsion for separations less than their diameter and zero otherwise. Using various colloid and polymer chemistry techniques, the interactions are tunable to include electrostatic, van der Waals forces, entropic forces, and steric forces. [33] Modifying any of these interactions could modify the concentration at which the suspension becomes jammed, and therefore, affect the glass transition.

The glassy dynamics of hard-sphere colloidal systems are examined quite extensively in previous studies. [28,34] While previous studies have reported a range of concentrations at which the onset of the phase changes occurs, it is generally accepted that the liquid phase occurs below a volume fraction of $\phi \approx 0.49$. Between $0.49 < \phi < 0.74$, the colloids may crystalize with a face-centered cubic (FCC) structure. If crystallization is avoided, for the concentration range $0.49 < \phi < 0.58$, the suspension shows dynamics comparable to the super-cooled phase. The glass transition occurs at $\phi_g \approx 0.58$ when the particle dynamics are sufficiently arrested due to jamming by their nearest neighbors. This is defined as “caging”, as particle motion is directly related to cages formed by neighboring particles.
1.2.2 Glass Fragility

As introduced in the previous section, the route from a liquid phase to a glass phase is an important process to understand during the production of glassy materials. [30-32] The rate of increase of dynamic properties, e.g. viscosity and relaxation time, is not universal of all glass-forming liquids as T approaches T_g for molecular glasses or as \( \phi \) approaches \( \phi_g \) for colloidal glasses. As first shown in the seminal work by Angell on molecular glasses, the “Angell Plot”, reproduced in Figure 2.2, certain glass formers exhibit an Arrhenius increase of viscosity with decreasing temperature while others exhibit a super-Arrhenius increase. [31] The differences in behavior are rooted in underlying intramolecular forces in the liquids. Certain liquids, such as SiO_2, have an extended network of directional covalent bonds, which may lead to the Arrhenius behavior. More complex molecular glasses, such as polymeric melts, tend to show super-Arrhenius behavior. There are also glass formers that show intermediate fragility behavior, such as glycerol. The rate of increase of viscosity for these glasses is somewhere between Arrhenius and super-Arrhenius.

One measure of the degree of temperature dependence for glass-forming liquids is to characterize the deviation from Arrhenius behavior. Angell proposed glasses could be categorized into two classes. A “strong” glass is a glass-forming material that exhibits an Arrhenius increase in viscosity while a “fragile” glass exhibits a super-Arrhenius increase. [32] In order to classify the two classes of glasses, a fragility index, \( m \), was introduced where \( m = \frac{d(\log_{10}\eta)}{d(T_g/T)} \bigg|_{T=T_g} \). Based on this classification method, strong glass-formers would have a smaller fragility index than fragile glass-formers as the rate of increase of viscosity with decreasing temperature becomes more gradual. Returning to
the Angell Plot, strong glass formers, e.g. SiO$_2$ and GeO$_2$, have a fragility index of $m=20$, while fragile glass formers, such as Toluene, have a fragility index greater than 100. [32]

The parallels between the fragility of molecular glasses shown in Figure 2.2 and concentration dependence of the relaxation time for microgel particles shown in Figure 2.1 should be immediately apparent to the reader. Therefore, it was suggested that the fragility phenomena observed in molecular glasses is also present in colloidal glasses. [23] Model hard-sphere systems have typically been considered fragile glass formers,
which transition from a liquid phase to a glass phase over a 5% change in volume fraction. Soft, deformable colloids exhibited a gradual increase in the relaxation time with increasing concentration and are consequently labelled as strong glass formers. It was also revealed that by tuning the elasticity of the microgel, a glass with intermediate fragility behavior is observed. Therefore, it was concluded that the fragility of a colloidal glass is directly related to its elasticity. [23]

1.2.3 Dynamic Heterogeneity

While the aforementioned definition of the glass transition entails the bulk process, it is important to consider the dynamics of the individual particles that form the glassy material. Developing connections between the microscopic processes and the observed phase change from a liquid to a solid will help develop a better understanding of the glass transition. Specifically, it is of interest to explain the differences in the microscopic processes between strong and fragile glass-forming liquids that lead to the differences in the rate of increase of the dynamic properties.

One prevailing theory regarding the microscopic world of the glass transition, the mode-coupling theory, generalizes the process into three steps based on the timescale of the particle motion. [25-28] The first step regards the very fast ballistic particle motion which occurs at very small timescales. The second step involves the rattling of the particles inside their “cages”, which are formed by the nearest neighboring particles. While the first step is a solo process, this step depends strongly upon the separation distance between particles and the dynamics of their nearest neighbors. This step is often regarded as the β-process. The final step entails how the particles escape from their cages,
which occurs at relatively long timescales. This is regarded as the \( \alpha \)-process, or the \( \alpha \)-relaxation process. \([25,28]\) It leads to the relaxation of particles as cages rearrange into new configurations. Therefore, typically the timescale at which this process occurs is regarded as the relaxation time for the material.

This theory describes the glass transition as a homogeneous process, which is contrary to many previous studies. Many studies have reported that the \( \alpha \)-relaxation process is strongly heterogeneous with growing dynamic length scales of cooperative molecular motion as a material approaches the glass transition. \([26,28]\) Super-cooled liquids exhibit spatially heterogeneous dynamics due to a varied distribution of particles in jammed and unjammed states, i.e. the motion of individual particles is impeded due to the presence of their nearest neighbors. In order for a jammed particle to become unjammed, the particles surrounding the jammed particle will also have to become un jammed. Consequently, mobile particles tend to form clusters that display cooperative motion. Similarly, clusters of immobile particles will also be observed throughout the material. \([26,28]\) The distribution of mobile and immobile clusters throughout the material will be strongly heterogeneous and will grow in size as the glass transition is approached.

A popular theory to explain this dynamic heterogeneity is the Adam & Gibbs (AG) Theory. \([25-28,34]\) This proposes the concept of cooperatively rearranging regions (CRR) which form as super-cooled liquids approach the glass transition. \([25-28,34-36]\) While this behavior was originally observed on molecular glasses, similar behavior was observed in colloidal glasses. \([29]\) Shown through equilibrium statistical mechanics, the AG theory describes the presence of dynamic heterogeneity as an enthalpy driven
process. Sub-regions in the liquid are able to rearrange their configurations independent of other sub-regions. [29] This reorganization process occurred through cooperative motion between neighboring particles leading to the formation of the CRRs. According to the AG theory, the configuration entropy, $S_{\text{conf}}$, of the CRRs is inversely proportional to the activation energy for relaxation of the material. Consequently, the growth of dynamic properties approaching the glass transition is directly related to the growth of the CRRs.

The AG theory directly relates the relaxation dynamics of glassy materials to the CRRs through the following relationship:

$$\tau = \tau_0 \exp\left(\frac{z\Delta G_A}{T}\right)$$

Here, $\tau$ is the relaxation time of the material, $\Delta G_A$ is the activation energy, and $z$ is the number of particles in the CRR. This relationship is directly associated to $S_{\text{conf}}$ of the CRRs through $S_{\text{conf}} = A \frac{z}{z\Delta G_A}$ where $A$ is the free energy. This leads to a connection between the relaxation time and $S_{\text{conf}}$.

$$\tau = \tau_0 \exp\left(\frac{A}{TS_{\text{conf}}}\right)$$

In addition to showing the connection between the relaxation dynamics and the dynamic heterogeneity, these equations also show the strong connection between temperature and dynamic heterogeneity.

While the AG theory proposed the existence of CRRs, it did not provide a definition for the nature of these regions or a method to identify them. Many recent studies, involving molecular simulations and colloidal experiments, have shown that particles in the CRRs tend to be composed of lesser groups of particles, which move in a string-like fashion. [28] The strings are elementary groups of particles, which make up the highly mobile clusters. They are defined by particle displacement. As one mobile particle moves
from point A to point B, another particle moves to position A to fill the void. This process can propagate to include many particles and can eventually reach a maximum when no particle fills the corresponding void. The strings behave as dynamic polymers in solution which readily form and disintegrate at equilibrium.

1.2.4 Glass Transition under Spatial Confinement

The previous sections discuss the glassy dynamics with a focus solely on glassy behavior in the bulk. However, many previous studies have shown a deviation from bulk behavior when super-cooled liquids are confined with at least one length scale on the order of several particle diameters. [37-41] Confinement is induced in several geometries found in industrial practices, such as micro-porous membranes and micro-fluidics, and therefore it is important to understand how it influences the glass transition. Previous studies have demonstrated that the glass transition of a confined liquid will increase, decrease or even remain unaffected. [37-39] Simulation studies showed that an increase in \( T_g \) results from strong interactions between the confining surfaces and particles near the surfaces. [40] Experimental work showed that controlling the surface interactions with the confined particles also had a strong effect on the glass transition. [40-41] Strong interactions between the confining surface and particles leads to an increase in \( T_g \) by slowing particle motion near the surface. When there are weak interactions between the confining surface and the particles, there is a decrease in \( T_g \).

Previous studies have shown that when a super-cooled liquid is confined to dimensions smaller than the characteristic size of the CRR, the glass transition will be induced. [42] Consequently, there is a direct relationship between the length scales
associated with confinement and the length scales associated with the CRRs. When the size of this length scale approaches the size of the confinement gap, the particle dynamics begin to deviate from bulk behavior. Therefore, the length scale associated with the confinement effect is considered to be the upper-bound of the length scale for dynamic heterogeneity. As specified in the previous section, the length scale of the CRRs grows as the temperature of glass-forming liquid is reduced approaching $T_g$. The confinement length is thus a temperature-dependent property that grows as the temperature of the liquid is reduced.

Results of simulation studies have directly linked glass fragility and the confinement effect. Looking at the temperature dependence of polymer melts, the fragility transitioned from a fragile to a strong glass-forming suspension as the liquid became strongly confined. [43] Interestingly, when observing dynamic heterogeneity in the melts, the strings of mobile particles showed little dependence on the confinement gap size. This is explained by the packing efficiency of the particles in the glass-forming material. At the highest degree of confinement, the packing frustration will be at its weakest, leading to a reduction in the glass fragility. Weak packing frustration will reduce the topological constraints that hinder particle mobility, leading to a more gradual transition into an arrested state. [25] As confinement tends to transition materials to strong glass-forming behavior, these effects will be more prominent in fragile glasses than in strong glass formers. This suggests that confinement will have a minimal effect on strong glass-forming liquids.
1.3 Dissertation Outline

As illustrated by this introduction, microgels have a broad range of uses, from biomedical applications to studies in fundamental physics. This dissertation details the experimental work investigating the interactions and dynamics of microgels in aqueous suspensions. Basic synthesis methods of microgels will be introduced in Chapter 2. The synthesis and analysis of microgels used in this work will be explained in detail with a strong emphasis on their thermal behavior.

In Chapter 3, it is shown that microgels can mimic the properties of lubricin, a globular protein, in the pursuit of designing a biomimetic super-lubricant. Mixtures of microgels and the biopolymer, hyaluronic acid, will be tuned to capture the unique properties of the synovial fluids, a naturally occurring lubricant found in the synovial joints of mammals. A rheological study is performed to show that these mixtures replicate the material properties of the synovial fluids. The interactions between microgels and the biopolymer are observed in situ through a microscopy study. In the last part of the chapter, a nano-tribology is performed to observe the low friction behavior of these mixtures grafted to a functionalized substrate.

Starting in Chapter 4, the glassy dynamics of microgel suspensions are probed to elucidate the differences between strong and fragile glass-forming liquids. A direct, three-dimensional microscopic study is performed to observe the dynamics of fluorescent microgels in dense suspensions. Using particle tracking algorithms, the coordinates and trajectories of each particle are measured in the suspension. Then, the concentration-dependent mobility and relaxation dynamics are analyzed for each microgel suspension.
It is demonstrated here that varying the microgel elasticity controls the fragility of the colloidal glass.

The analysis of glass fragility is continued in Chapter 5. Here the heterogeneous dynamics of glass-forming liquids are investigated by examining clusters of mobile and immobile particles. A dynamic length scale associated with the CRRs is calculated and their growth is shown to be strongly dependent on the glass fragility. The nature of these CRRs is analyzed and it is confirmed that particles move in a string-like fashion. As with the dynamic length scale, the average string length is strongly dependent upon the glass fragility, and string sizes are shown to be longer in strong glass-forming liquids than in fragile glass formers.

In Chapter 6, it is examined how strong glass-forming behavior deviates from bulk behavior as the material is confined. It is shown that strong glass formers remain mobile in the super-cooled phase at confinement length scales, which typically induce the glass transition for fragile glass formers. It is suggested that soft microgels deform at small confinements, which prevents a confinement-induced glass transition. Future work in this area is discussed as the investigation will be advanced to study the confinement effect on glass-forming suspensions of intermediate fragility. This will hopefully lead to a deeper understanding of the confinement effect. Finally, in Chapter 7, a summary of the dissertation is provided with a perspective of the future work in the field of studying the dynamics of dense microgel suspensions.
CHAPTER 2
SYNTHESIS & ANALYSIS OF MICROGEL PARTICLES

2.1 Introduction

Now that the broad range of applications and properties of microgel particles has been introduced, the aim of this chapter is to detail the experimental procedures for creating the microgel structure. The methodologies for microgel synthesis are discussed here with a strong emphasis on emulsion polymerization, the technique predominantly used to synthesize the microgels used throughout this dissertation. The criterion for selecting polymer backbones for the microgel structure is also discussed here, with an emphasis on the importance of biocompatibility. The chapter concludes by detailing the synthesis procedures used for this dissertation and how thermal analysis reveals the solution behavior of the microgel particles.

2.2 Review of Microgel Synthesis Methods

There are four common methods reported in previous studies to create microgel particles: emulsion polymerization, anionic copolymerization, crosslinking of neighboring polymer chains, and inverse micro-emulsion polymerization. [44-48] An
emulsion polymerization involves the emulsion of a monomer, a surfactant, and an initiator. [45] Typically, the continuous phase is water and the discontinuous phase is an oil. Small latex particles form in the initial phase of the reaction, which act as macro-monomers, and then they are linked together to form the microgel structure. Anionic polymerization is an additive polymerization method where the polymer chain forms in three steps: initiation, growth, and termination. [46] A crosslinking agent connects the polymer chains into the microgel structure. The third method, crosslinking of neighboring polymer chains, involves crosslinking previously synthesized polymers together to form a colloidal structure. [47] An inverse micro-emulsion polymerization is similar to an emulsion polymerization involving a micro-emulsion where the discontinuous phase is typically water and the continuous phase is an oil. [48]

The method employed for this work is the emulsion polymerization method. [44-45,49-50] It is preferred predominantly due to strong control over the microgel size and a low degree of polydispersity. One of the most common methods is surfactant free emulsion polymerization, SFEP. [44,49] This method utilizes free radical polymerization to form polymer chains. This entails propagation growth of the polymer facilitated by an initiator molecule which radicalizes the monomer units. This is a facile method for creating microgels as it is a “one pot” method, where the entire particle is formed in a single batch process. Without the presence of a surfactant during the synthesis, the microgels are less likely to contain any residual contamination. To form the complex network of a hydrogel, a crosslinker molecule is incorporated during the synthesis which replaces a monomer unit on two polymer strands, connecting the polymers together.
Another subset of emulsion polymerization employed to create monodisperse microgels is dispersion copolymerization. [50-52] Similar to free radical polymerization, this is also a one-pot synthesis method. It is a type of precipitation polymerization, where the monomer and initiator are completely soluble in a continuous phase but the resulting polymer is insoluble. The size of the discontinuous phase droplets in the continuous phase as well as the amount of monomer added to the synthesis will play a significant role in governing the size of the polymer macro-monomers. [51-52] The polymers act as the macro-monomers in the discontinuous phase and aggregate together early in the polymerization process forming a spherical structure. These spheres will grow into a particle with sizes on the order of a micron. Typically, a stabilizer polymer is grafted onto the surface of the sphere. This prevents aggregation of the microgels in the dispersing liquid. [51-52]

2.3 Experimental Synthesis

2.3.1 Polymer Selection

Since many potential applications for microgels relate to biomedical purposes, it is desirable for the polymer backbone of the microgel to be biocompatible. A fully biocompatible material must inflict no short-term effects, e.g. cytotoxicity, nor long-term effects, e.g. carcinogenicity. [53] One polymer which has garnered a lot of interest recently is Poly(N-isopropylacrylamide) (PNIPAM). [54-55] Although its carcinogenicity is still under investigation, it is widely regarded as biocompatible. [56] It is also one of the most widely studied responsive polymers, and it has a lower critical solution temperature (LCST) of ~32°C in water where it experiences a swollen-to-collapsed
transition with increasing temperature. [57] It experiences this coil-to-globule transition due to interactions between the hydrophobic isopropyl group and the hydrophilic amide group. This behavior parallels the cold renaturation of small globular proteins, making it favorable for biomimicry applications. [58-59] Below the LCST, the PNIPAM chains readily form intermolecular hydrogen bonds causing the particles to swell. Above the LCST, the polymer chains form intramolecular hydrogen bonds and the particles collapse. This strong hydrophobic nature leads to the self-assembly of particle aggregates at high temperatures. [57-58] Therefore, these microgels must be stabilized in solution to prevent the formation of particle aggregates above the LCST by adding a charge to the particle or by introducing a surfactant to the system. Based on the degree of crosslinking, the size and transition temperature are tunable. [57] At high degrees of crosslinking, the particle experiences minimal size change across the LCST and can be considered hard-sphere-like below the LCST. At low degrees of crosslinking, the microgels become highly deformable below the LCST.

One method to develop highly biocompatible polymers is to turn to biopolymers already found in nature. Polymers based on methacrylate monomers combine components of molecules found in the cell membrane. [50,61-62] These polymers are non-biofouling that resist nonspecific protein adsorption. This typically prevents the adhesion of infection-causing bacteria to the polymer. If used for a biomedical purpose, the transport of an infection would greatly hinder the purpose of the material. Therefore, biofouling materials are not considered biocompatible and not often used for biomedical purposes. The monomer chosen for this work is [2-(Methacryloyloxy)ethyl]-dimethyl-(3-sulfopropyl)] ammonium hydroxide (MSA). PolyMSA (PMSA) polymers are hydrophilic
and zwitterionic which are ideal to functionalize for biomedical applications. The dual charges are attractive for variety chemical modifications. However, this charge behavior may lead to aggregation in solution at room temperature and an upper critical solution temperature (UCST) is expected.

2.3.2 PNIPAM Microgel Synthesis

PNIPAM microgel particles are synthesized using the method of free radical polymerization described above. [49] As PNIPAM is insoluble in water above 32°C and NIPA is soluble, it is an ideal candidate for SFEP polymerization method to form the microgel structure. Water will act as both the continuous phase and the discontinuous phase. For these studies, N-isopropylacrylamide (NIPA) is purchased from Acros Organics and purified to remove any inhibitor via recrystallization. N,N’-methylenebisacrylamide (BIS), the crosslinker, and potassium persulfate (KPS), the initiator, are purchased from Sigma-Aldrich and used as received. The polymerization reaction is performed at 60°C and under a N₂ purge. It is initiated using KPS to start the creation of polymer chains. Each polymer chain is capped with a persulfate group, resulting in slightly negatively charged PNIPAM polymers. As the polymer chain grows in the solution, it will reach a critical point where the polymer is no longer soluble at the reaction temperature. At that point, the polymer will drop out of solution and it ceases to continue to grow in length. The PNIPAM hydrogel structure forms by crosslinking the polymers together with BIS. As NIPA is an uncharged monomer, a co-monomer is typically required to create a modifiable polymer. To create a fluorescent microgel particle, the co-monomer 2-aminoethyl methacrylate (AEMA), which carries a positive
charge, was included in the synthesis. A fluorescent dye, NHS-Rhodamine, was attached to AEMA monomer prior to synthesis and created microgels that absorb green visible light (552nm) and emit orange-red visible light (575nm).

2.3.3 PMSA Microgel Synthesis

PMSA particles are synthesized via dispersion polymerization as described in the previous section. [50] The monomer, MSA, is purchased from Sigma-Aldrich and used as received. The reaction is performed in a mixture of 80 vol% hexane and 20 vol% water and initiated with 2,2’-Azobis(isobutyronitrile) (AIBN). Ethylene glycol dimethacrylate (EGDMA) is used as a crosslinker and poly(ethylene glycol) methacrylate is used as a reactive steric stabilizer. The resulting PMSA hydrogel suspensions are purified via dialysis for two weeks at room temperature using a SpectraPor® dialysis membrane with a diameter of 22 mm and a flat width of 34 mm and finally re-dispersed in fresh deionized water before characterization. The resulting PMSA hydrogel suspension is concentrated via repeated centrifugation at 9000 g for 45 min using a Fisher Scientific AccuSpin 400 and re-dispersed in deionized water to desired concentrations.

2.4 Thermal Behavior of Microgel Solutions

2.4.1 Solution Properties Determined from Size and Polydispersity Analysis

The size and size distribution of PNIPAM and PMSA microgels particles in dilute aqueous suspensions are determined by light scattering (Brookhaven ZetaPALS), as shown in Figure 2.1. At room temperature, the hydrodynamic diameter ($d_H$) of the PNIPAM particles is measured to be 760 nm with a narrow polydispersity of 6%. The
characteristic LCST behavior of PNIPAM particles is confirmed by its strong temperature (T) dependence of particle swelling as shown in Figure 2.1(a). As T increases from 25°C to 40°C, the size of swollen PNIPAM particles in water gradually decreases, indicating the LCST range of T=26–39°C is consistent with the range reported
in the literature. [57-58] The size reduction of ~30% as T exceeds the LCST range indicates that the PNIPAM microgels used in this work can be regarded as hard-sphere particles. [23] The size and size distribution of PMSA microgels in aqueous suspensions of T=25°C to 65°C is shown in Figure 2.1(b). A notable reduction in both PMSA particle size and polydispersity is observed as T exceeds 55°C, confirming the UCST behavior of PMSA microgel particles in aqueous suspensions where these microgels form aggregates at room temperature but become dispersed at T> UCST ~ 55°C. The actual size of dispersed PMSA microgel particles is estimated to be d= 750 nm at T= 65°C. Therefore, the d_H of both PNIPAM and PMSA microgels is approximately identical.

2.4.2 Microgel Elasticity Revealed from Thermal Analysis

Next, it is tested how the amount of crosslinker added to the synthesis affects the elasticity of PNIPAM microgels. For this analysis, fluorescent PNIPAM microgels are synthesized using the procedure described in the previous section and have an average d_H of 1.66 μm with a standard deviation of 0.25μm. The crosslinking amount is defined by the crosslinker (CL) ratio, CL = \([BIS]/[BIS]+[NIPAM]\) , where [BIS] and [NIPAM] are the mass concentrations of BIS and NIPAM, respectively, added to the synthesis. It is expected that CL ratio of PNIPAM microgels affect the LCST behavior of PNIPAM microgels. [23] Five CL ratios are examined in order to observe a range of particle deformability. These values are CL=0.9%, 1.6%, 2.2%, 3.3%, and 6.6%. The microgel stiffness is first observed through the size change of d_H across the lower critical solution temperature, LCST, of PNIPAM, shown in Figure 2.2. In water, the swollen PNIPAM particles collapse as the temperature, T, increases from 25°C to 40°C. This collapse occurs as T
increases past the LCST of PNIPAM, \( \sim 32^\circ \text{C} \), indicating an LCST range of \( T = 26 \) – 39\( ^\circ \text{C} \). Again, this behavior is consistent with previous studies on PNIPAM microgels. [57-58]

As expected, decreasing the CL ratio results in a greater size change and it is projected that smaller CL ratios will lead to more deformable particles.

2.5 Conclusion

The stimuli-responsive microgel particles based on PNIPAM and PMSA will be employed in Chapter 3. Due to their solution behavior, biocompatibility, and tunable properties, these particles will be used to replicate the properties of globule proteins. The PNIPAM microgels of variable elasticity are analyzed in the remaining chapters of this
dissertation. Since these microgels behave as model colloidal particles, they are used to probe particle dynamics in glassy colloidal suspensions. Due to their size, these microgels can be observed using a microscopic study and the particle dynamics can be measured through particle tracking techniques.
3.1 Introduction

In this chapter, the applicability of microgels is demonstrated through the design of a bio-inspired super-lubricant. The inspiration for this project is the synovial fluids, a superior lubricant found in mammalian synovial joints, which have a typical life span of 40-80 years. This lubricant is a complex fluid comprised of a biopolymer, hyaluronic acid, and globule proteins. It is shown here that this intricate mixture found in the synovial fluids is replicated by replacing the globule protein with a synthetic microgel particle. The bulk rheological properties and the low frictional behavior is reproduced by tuning the aqueous network of mixtures of microgels and biopolymers. It is also confirmed by light scattering and fluorescence microscopic characterization that added microgel particles can enhance the HA network by hydrogel-mediated hydrogen bonding, leading to the fractal HA-hydrogel aggregating networks in aqueous suspensions.
3.2 Background

Synovial fluids (SF) are a superior biological lubricant present in freely moving mammalian synovial joints. [63-67] It has a nearly life-long working cycle and an ultralow friction coefficient in the range of 0.001-0.01 under fast changing loading conditions, providing far greater lubrication than most synthetic oil and aqueous based lubricants. [67-70] Such super-lubricity is debated to result from a complex network among sodium hyaluronate (NaHA), which is the most abundant polymeric component in SF, phospholipid membranes, and lubricin and other proteins. NaHA is the sodium salt of hyaluronic acid (HA), which is a polysaccharide with a high molecular weight naturally present in mammalian bodies. [71-77] HA molecules form a highly viscous mesh network due to entanglements between polymer chains caused by hydrogen bonding. [74] Importantly, HA has been used as visco-supplementation to alleviate the joint pain [78]. Yet it is now widely accepted that a simple aqueous solution of only NaHA cannot bear the high loads applied by most joints, until it forms a complex matrix with phospholipid membranes, lubricin, or both. [72,79] Lubricin, which consists of polypeptide-based glycosylated mucinous domains, can form large scaled aggregates due to depletion flocculation caused by the high molecular weight NaHA chains in SF as well as due to association between hydrophobic regions of the globule protein. [72,80] Despite the debated contribution of phospholipid-HA and lubricin-HA aggregation to the super-lubrication of SF, the supramolecular association of HA chains with other macromolecules is critical to maintain sufficient viscoelasticity of SF and promote the lubrication performance. It is thus expected that such complex aggregates are present under both quiescent and vastly varied shearing conditions associated with different joint
motions through combined mechanisms of both hydrodynamic and boundary lubrications. The hydrodynamic lubrication is indicated from the shear-dependent properties of aqueous solutions of NaHA, which shows shear thinning and an elastic response at shear rates comparable to that of joint motion with relative thickness NaHA films under low load. At high loads, supramolecular aggregates of HA with other macromolecules in SF can be trapped at cartilage surfaces to effectively dissipate the energy due to locomotion of the joints [73-76] and prevent the contact between moving joint surfaces. [77,81]

Advancement in understanding the super-lubrication mechanisms of SF has paved the foundation in biomimetic design of an artificial biolubricant for biomedical ramification as well as many engineering practices. In molecular design of an efficient biolubricant, it is critical to control the interaction of multiple components in the complex fluids to form intermolecular or inter-particle aggregation layers at interface that can function distinctly from the individual components alone. [72,79] It is also suggested that highly hydrated hydrogen bonded interfacial aggregates could be promising aqueous based lubricants to increase load-bearing capacity and lower friction by strong hydration. However, due to the synergy of multiple components of different molecular length scales in the SF for the ultimate efficient lubrication, it remains difficult in synthesizing and assembling simple polymeric solution to mimic all the functionalities exhibited in natural SF. In this chapter, the aggregation of HA chains with biocompatible microgel particles in aqueous suspensions is investigated as biomimetic artificial biolubricants. In this biomimetic approach, the network structure of NaHA is enhanced through the addition of microgel particles, which are cross-linked polymer chains in spherical particle shape to mimic the
structural characteristics of globule proteins and phospholipid vesicles through folding induced by inter- and intramolecular hydrogen bonding. It is hypothesized that these particles can provide the “ball-bearing” like effect necessary for artificial biolubricants. Specifically, a focus is placed on stimuli-responsive hydrogels, PNIPAM and PMSA particles, in mixtures with HA chains in aqueous suspensions in this work. [50,54-55] At room temperature, below the LCST, PNIPAM chains in a microgel particle readily form intermolecular hydrogen bonds leading to swollen particles and thus it is expected that PNIPAM can also form hydrogen bonds with HA. [54-55] Above the LCST, PNIPAM chains form intra-molecular bonds and the particles will collapse and self-assemble into aggregates. The contrasting solution behavior of PNIPAM and PSMA microgel particles interacting with HA can also provide insight to the nature of HA-hydrogel complex formation and associated viscoelastic properties. Additionally, the size and transition temperature of both microgel particles are tunable by the degree of crosslinking. At high degrees of crosslinking, the particle experiences minimal size change across the LCST and is considered hard-sphere-like. Hard-sphere particles are less susceptible to shear and would be a more effective replacement for globule proteins and phospholipid vesicles to improve lubrication performance.

In this chapter, the structure, rheological and frictional properties of NaHA and microgel particle mixtures in aqueous suspensions are examined of varied HA and microgel particle concentrations. Both the linear and nonlinear rheological regimes are probed to assess the viscoelasticity of these biomimetic complex fluids in comparison to that of healthy SF to determine the optimal concentration ranges. The microstructures of the mixtures in aqueous suspensions are directly characterized by fluorescence
microscopy to further determine the interactions between HA and microgel particles. The low friction performance of the mixtures at optimal concentration conditions, at which the rheological properties of HA-microgel particle aggregates are comparable to that of healthy SF, is further investigated by atomic force microscopy (AFM).

3.3 Experimental

3.3.1 Materials

PNIPAM microgel particles are synthesized using the method of free radical polymerization and PMSA particles are synthesized via dispersion polymerization, both synthesis methods and analysis are discussed in the previous chapter. [49-50] NaHA (molecular weight, Mw= 540 kDa), is purchased from Sigma-Aldrich and used as received. Fluorescence-labeled HA, hyaluronate rhodamine (Mw= 350 kDa, 5mol% fluorescence label substitution), is purchased from Creative PEGWorks and used as received. Bovine SF are purchased from Animal Technologies, Inc, in which the concentrations of HA and lubricin in bovine SF are reported to be 1mg/ml and 0.1mg/ml, respectively. [82] Glycidyl methacrylate (GMA) is purchased from Sigma-Aldrich and purified by basic alumina column before use. Polyglycidyl methacrylate (PGMA) is synthesized by dispersion polymerization and used to functionalize solid substrates to accommodate strong attachment of hydrogel-HA mixture for friction measurement. [83]

3.3.2 Rheological Measurements

A stress-controlled rheometer (Malvern Bohlin Gemini HRnano) with a cone-and-plate fluid cell of 40 mm in diameter and 4° in cone angle is employed to study the linear and
nonlinear viscoelastic properties of hydrogel-HA mixtures in aqueous suspensions. During the measurements, the gap distance between the plates is set constant at 100μm and the temperatures is held constant at 25°C using a Peltier Element. After the sample is loaded and the desired gap size is obtained, a thin film of silicon oil (Aldrich) is carefully applied to the menisci of the sample cell in order to minimalize evaporation and thereby allow reliable measurements over several hours. The Newtonian viscosity of the mixture suspensions is measured at a constant low stress that is determined to be proportional to the applied shear strain. A frequency range of $\omega \approx 0.063$-125.7 rad/s is applied to the sample at a constant strain, $\gamma = 0.5\%$, which is preliminarily determined to ensure the linear shear response. To examine the non-linear behavior, a strain range of $\gamma = 1\%-2000\%$ is applied to the sample at constant $\omega \approx 6.3$ rad/s.

3.3.3 Microscopic Characterization and Image Analysis of HA-Microgel Mixtures

The microstructure of PNIPAM and PMSA hydrogels mixed with 10 wt% Rhodamine-labeled HA is visualized using confocal laser scanning microscopy (Zeiss LSM 5 Pascal, 100x objective, oil immersion, NA=1.4). A home-built sample chamber is attached to a coverslip using UV-curing optical adhesive (Norland 80) to seal the suspension to minimalize evaporation. Approximately 200μL of the sample is inserted into the chamber and allowed ~5 hours to acquiesce to an equilibrium state. The microstructure of PNIPAM hydrogel-HA mixtures is also characterized using scanning electron microscopy (SEM) (Magellan 400 Field Emission). The mixture is deposited on an SEM sample plate and dried in the hood for 12 hours. The dried mixture is then coated
with a thin layer of iridium of 7 nm thick. SEM micrographs are acquired at an acceleration voltage of 3.0 kV without causing any noticeable damage on the sample.

3.3.4 Local Friction Measurement

HA thin films with and without added PMSA particles are grafted to a PGMA pre-treated solid surface. PGMA thin layer grafted to a silicon substrate provides a universal coating surface to enhance the grafting of HAs or HA-PMSA mixture to the substrate for reliable and repeatable friction measurement. Briefly, a dilute solution of PGMA in chloroform is spin coated (Laurell Technologies, WS-400BZ-6NPP/Lite) at 4000 rpm for 60 s and thermally annealed for 1 hour at 140°C. Excess polymer is removed by sonication in acetone for 20 min. Next, HA polymers or HA-PMSA hydrogel mixtures are deposited on the PGMA treated substrate and spin coated at 4000 rpm for 60 s and thermally annealed for 16 hours at 170°C. Excess hydrogel and HA is removed by sonication for 20 min in acetone.

The surface morphology and local friction of grafted HA polymer layer and mixed HA-PMSA layer mixture in PBS solution are characterized by tapping-mode and friction-mode atomic force microscopy (AFM; Veeco, Multimode) using a silicon tip (Veeco) performed over a scanning area of 10 μm x 10 μm, respectively. Frictional micrograph image analysis examines the microtribological behavior of HA and HA-PMSA films. The friction force on the AFM tip is estimated by the difference between the trace and retrace voltage signal. This frictional force, $F_L$, is proportional to the normal load of the AFM tip following the Amonton’s law that predicts the ratio of $F_L$ to $F_N$ yields the local friction coefficient, $\mu$. 


3.4 Results & Discussion

To start, the zero shear viscosity of NaHA aqueous suspension is examined for increasing NaHA concentration from 5-30 mg/ml with and without added microgel particles in comparison to the reported viscosity of natural SF, as shown in Figure 3.1. [30] It is expected that the viscosity of microgel particle-free NaHA aqueous solutions increases from 0.6 Pa·s to 10 Pa·s exponentially with HA concentration. [73-76] The comparable viscosity of simple HA solution to that of healthy SF, in which the HA

![Figure 3.1: The zero-shear viscosity, \( \eta_0 \), against HA concentration in aqueous suspensions of without added microgel particles (black squares), added with PNIPAM microgel particle of concentration \([\text{PNIPAM}] = 15 \text{ mg/ml}\) (green triangles), 30 mg/ml (inverted blue triangles), and added with PMSA particles of \([\text{PMSA}]=17 \text{ mg/ml}\) (red circles), in comparison to that of healthy human SF reported in the literature (orange diamonds) and bovine SF measured in this work (maroon stars). All the samples are measured at a constant stress previously determined to be in the Newtonian regime and at constant room temperature.]

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concentration is about 2.5mg/ml or 0.36μM, is found at the HA concentration about 15-20mg/ml, equivalent to 25-40μM. The disparity between the concentrations of HA in natural SF and the HA solutions of this work is possibly due to the difference in the Mw of HA in these two systems. The Mw (540kDa) of NaHA used in this work is more than 10 times lower than that of HA in SF typically in the range of Mw=6.3-7.6MDa, thus the HA concentration in this work needed to achieve the solution viscosity to that of natural SF should be higher. [85] It is known that the increased entanglement of polymer chains in solution can lead to the viscosity increase. To compensate for the difference in HA Mw between HA solutions in this work to that of natural SF, the HA concentration is rescaled to k*[HA]=0.2-0.4μM, where \( k = (M_{w,HA}/M_{w,SF})^{9/5} \) by considering the hydrodynamic radius, Rg of HA chains in the aqueous solution following the scaling behavior of Rg \( \sim M_w^{9/5} \). The viscosity of bovine SF is also measured, in which the HA concentration is reported to be about 1mg/ml, to be 0.15Pa·s, which is considerably lower than that of plain HA solutions used in this work as well as that of natural human SF. [82] Importantly, with added PNIPAM or PMSA microgel particles in the HA solutions, the viscosity of healthy SF could be achieved at reduced HA concentrations in the hydrogel-HA mixed suspensions as shown in Figure 3.1. For instance, at the HA concentration greater than about 15mg/ml, adding PNIPAM particles at the concentration of 15-30mg/ml can approach values within the range of the reported viscosity of human SF. Also at the same HA concentration, doubling PNIPAM particle concentration from 15mg/ml to 30mg/ml can lead to the viscosity enhancement by a fold of 2-4. Adding PMSA to the HA solution at room temperature seems to be less effective than adding
PNIPAM particles, possibly due to weaker PMSA-HA interaction than PNIPAM-HA interaction as further discussed below.

The optimal microgel particle concentrations to achieve desirable rheological properties of HA-microgel particle mixed suspensions are thus explored by the linear and non-linear viscoelastic spectra. The rheological properties of HA-microgel particle mixed suspensions depend on not only the HA concentration, but also on the interaction between HA polymers and microgel particles and their resulting aggregation structures. To elucidate the viscosity enhancement by the added microgel particles, the linear viscoelastic properties of HA-PNIPAM and HA-PMSA mixtures are examined by oscillatory strain measurements in comparison to that of natural SF. As shown in Figure 3.2, the frequency, $\omega$, dependent elastic and viscous moduli, $G'$ and $G''$, respectively, are measured at a constant strain, $\gamma=0.5\%$, at which the linear response is ensured. The linear viscoelasticity of HA-PNIPAM mixed suspensions shown in Figure 3.2a appear similar to the previously reported rheological behavior of healthy SF as well as plain HA aqueous solutions. [73] For all the varied HA concentrations, the linear shear spectra displayed the typical viscoelastic behavior of a Maxwellian fluid characterized by a crossover frequency, $\omega_c \approx 6.3$rad/s and 0.7rad/s for HA concentration, [HA]=15 mg/ml and 30 mg/ml, respectively. At low frequency, $G''$ is greater than $G'$, indicating predominantly viscous fluid of HA aqueous suspensions solution for both cases with or
Figure 3.2: Linear viscoelastic spectra of measured elastic moduli, $G'$ (solid symbols) and viscous moduli, $G''$ (open symbols) as a function of radian frequency, $\omega$ measured at 25°C for (a) PNIPAM-HA mixed aqueous suspensions of (i) [HA] = 10 mg/ml with added PNIPAM particles of [PNIPAM] = 0 mg/ml (red circles), 15 mg/ml (green triangles), and 30 mg/ml (inverted blue triangles), (ii) [HA] = 20 mg/ml with added PNIPAM particles of [PNIPAM] = 0 mg/ml (dark cyan diamonds), 15 mg/ml (purple left triangles), and 30 mg/ml (dark yellow right triangles) in comparison to those of bovine SF (black squares), and (b) PMSA-HA mixed aqueous suspensions of (i) [HA] = 10 mg/ml with added PMSA particles of [PMSA] = 0 mg/ml (red circles), and 17 mg/ml (green triangles), (ii) [HA] = 17 mg/ml with added PMSA particles of [PMSA] = 0 mg/ml (inverted blue triangles) and 17 mg/ml (dark cyan diamonds). All samples were measured at a low strain, $\gamma = 0.5\%$, to ensure a linear rheological response.
without added PNIPAM hydrogels. At $\omega > \omega_c$, $G'$ becomes dominant, possibly resulting from the HA-PNIPAM complex formation. [75] No clear decrease in $G''$ is observed, suggesting a broad spectrum in the relaxation of HA and HA-PNIPAM complex aggregates. At the same HA concentrations, the addition of PNIPAM microgel particles has led to a notable increase in the moduli, suggesting the formation of HA-PNIPAM complexes. It is noted that adding PNIPAM particles on modifying the viscoelasticity of the suspension of HA concentration becomes less effective than that of low HA concentration, suggesting the saturation of the HA-PNIPAM complex formation. Similarly as shown in Figure 3.2b, adding PMSA particles to HA suspension leads to the increase in both $G'$ and $G''$ of HA suspension. The viscoelasticity of HA-PMSA mixed suspension is greater than either plain PMSA hydrogel suspensions or HA solutions of the same concentration, suggesting the formation of HA-PMSA complexes. Because of the known UCST behavior of PMSA, the mixture of HA and PMSA at room temperature exhibits the gel like behavior with a characteristic scaling of both $G'$ and $G''$ with $\omega^{0.5}$. Similar to the effect of added PNIPAM particles, the effect of PMSA on modifying the viscoelasticity of HA suspension also diminishes at high HA concentration. It is interesting to observe that despite the distinct solution behavior of PNIPAM and PMSA microgel particles at room temperature, both neutral hydrophilic PNIPAM and zwitterionic partial hydrophobic PMSA particles can interact with HA to form complex aggregates of enhanced viscoelasticity. Thus, the interaction between microgel particles and HA is hydrogen bonding dominant.
Figure 3.3: (a) Crossover frequency, $\omega_c$, (b) crossover moduli, $G^*$, and (c) structural unit, $\nu$, plotted against HA concentration, which are obtained from the linear shear spectra shown in Figure 2 for aqueous suspension of (i) without added microgel particles (black squares), added microgel particles of $[\text{PNIPAM}] = 15$ mg/ml (red circles) and 30 mg/ml (green triangles), and $[\text{PMSA}] = 17$ mg/ml (inverted blue triangles), in comparison to the reported literature values of healthy SF (olive hexagons) and our experimental results of bovine SF (maroon stars). The results of both SF samples are scaled by $k = (M_{w,HA}/M_{w,SF})^{9/5}$. 

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To determine the optimum concentrations of HA and microgel particles in the mixed aqueous suspensions, the crossover frequency, $\omega_c$ and crossover moduli, $G^*$, is summarized for values at which $G'$ and $G''$ are equivalent at $\omega_c$, against HA concentration, as shown in Figure 3.2(a-b), respectively. For both HA-PNIPAM and HA-PMSA mixtures, it is observed that increasing the concentration of HA leads to an exponential decrease in $\omega_c$, following Maxwell’s prediction of a viscoelastic liquid containing entangled polymer network. [27] It is thus suggested that the added microgel particles could further enhance the network formation of HA polymer in solution by functioning as crosslinkers possibly via hydrogen bonding. In comparison to the reported $\omega_c$ of natural SF, these results confirm that at [HA]=15-30 mg/ml, the obtained $\omega_c$ of the HA-hydrogel mixture can match within the range of the reported values of healthy SF. With the addition of microgel particles, a uniform increase of $G^*$ is observed at all concentration of HA. It is noted that the measured $G^*$ for the HA-hydrogel mixtures is far greater than that for healthy SF at all the HA concentrations. By fitting the obtained $G^*$ with the known relationship $G^* \sim \nu k_B T$, where $\nu$ is considered as the number of “complex structural units” per unit PNIPAM volume, $\nu$ normalized by number concentration of HA molecules, is plotted against HA concentration in Figure 3.2(c). [27] For the plain HA solutions without added hydrogels, $\nu$ can be simply considered the concentration of HA molecules and the increase in $G^*$ is resulted from the enhanced HA polymer network with increased HA concentration. The influence of added microgel particles on the HA network structure is also manifested in the increase of $\nu$ with HA concentration at given constant hydrogel concentrations. For instance, at [PNIPAM] = 15mg/ml and 30mg/ml, $\nu$ is increased by ~23% and ~47% from that for plain HA, respectively, suggesting a strong
interaction between HA and PNIPAM. Considering that the volume ratio of one microgel particle to an HA coil scales with \((R_H/R_{HA})^3 \sim 200\), where \(R_H\) is the measured radius of dispersed microgel particles in HA-free aqueous suspension and \(R_{HA}\) is the radius of swollen HA coils in aqueous solution and estimated based on the known HA molecular weight, it is estimated that by simple volume effect, the addition of particles at \([PNIPAM] = 15\) and \(30\text{mg/ml}\) can lead to an increase of \(2000\) and \(10000\) per \(\mu\text{m}^3\) structural units, or roughly a \(15\%\) and \(43\%\) increase in \(\nu\), respectively, in good agreement with these experimental results.

The mechanical strength of the hydrogel-induced HA network is also assessed by large amplitude oscillatory strain sweep measurements at a fixed frequency of \(\omega \approx 6.3\) rad/s as shown in Figure 3.4(a-b) for HA-PNIPAM and HA-PMSA mixed aqueous suspensions, respectively. Apparently, all the suspensions exhibit the linear viscoelastic regime with predominant \(G''\) over \(G'\) at small strain, \(\gamma < 1\%\). At higher \(\gamma\), the nonlinear regime is observed when \(G'\) exceeds \(G''\) and both \(G'\) and \(G''\) decrease steadily over at least one decade increase of strain. For all the HA-hydrogel mixed suspensions, the addition of microgel particles can clearly enhance the viscoelasticity from that of plain HA solutions, although it does not prevent the deformation and shear thinning of HA-hydrogel mixed suspension at high strain. Desirably for the lubrication benefit, the critical \(\gamma\) for the transition from linear to non-linear viscoelastic behavior shifts to higher values with added microgel particles than that in hydrogel-free HA solution.

To examine the interaction of HA with added microgel particles, the zeta-potential, \(\zeta\), of dispersed PNIPAM particles is examined in aqueous suspensions with added HA as
Figure 3.4: Non-linear viscoelastic spectra of $G''$ normalized by $\omega$ as a function of shear strain, $\gamma$ at 25°C for (a) PNIPAM-HA mixed aqueous suspensions of (i) $[HA] = 10$ mg/ml with added PNIPAM particles of $[PNIPAM] = 0$ mg/ml (red circles), 15 mg/ml (green triangles), and 30 mg/ml (inverted blue triangles), (ii) $[HA] = 20$ mg/ml with added PNIPAM particles of $[PNIPAM] = 0$ mg/ml (dark cyan diamonds), 15 mg/ml (magenta left triangles), in comparison to those of bovine SF (black squares), and (b) PMSA-HA mixed aqueous suspensions of (i) $[HA] = 10$ mg/ml with added PMSA particles of $[PMSA] = 0$ mg/ml (red circles), and 17 mg/ml (green triangles), (ii) $[HA] = 17$ mg/ml with added PMSA particles of $[PMSA] = 0$ mg/ml (inverted blue triangles) and 17 mg/ml (dark cyan diamonds). All samples were measured at constant $\omega = 6.3$ rad/s.
shown in Figure 3.5. No such measurement is conducted with PMSA particles with the concern of some possible PMSA aggregation in aqueous solutions at room temperature because of its UCST characteristics. The initiator, potassium persulfate that is used for the synthesis of PNIPAM particles leads to the net negative surface charge of PNIPAM particles with the measured $\zeta = -25$ mV in deionized water. The strong HA-PNIPAM interaction is confirmed by the increased negativity of PNIPAM particles with the increased concentration of HA added in the mixed aqueous suspension, suggesting the adsorption of HA chains on PNIPAM particle surfaces. Because NaHA is negatively charged in deionized water, the account of electrostatic interaction is excluded for the strong HA-PNIPAM interaction. Also the van der Waals interaction between HA and PNIPAM can be negligible due the nearly matched index of reflection of PNIPAM.
particles that contain approximately 97% water with that of aqueous medium. Therefore, the strong HA-PNIPAM interaction that results in the viscoelastic enhancement of the HA suspension is likely due to the intermolecular hydrogen bonding between HA and PNIPAM. Because of the distinct LCST and UCST characteristics of PNIPAM and PMSA microgel particles, the hydrophobic interaction is expected not to be the main driving force for the observed interaction between HA and microgel particles and could be arguably present only for the HA-PNIPAM complex formation. Thereby the similar hydrogen bonding to the HA-PMSA interaction contributes to the viscoelastic enhancement in HA-PMSA mixture.

The structure of HA-hydrogel complexes in aqueous solutions is characterized directly by fluorescent microscopy using 10 wt% fluorescence labeled HA of similar Mw to that of plain HA-hydrogel mixed suspensions, as shown in Figure 3.6(a-b) for HA-PNIPAM and HA-PMSA cases. It should be noted that the rheological behavior of Rhodamine fluorescence-labeled HA solution with and without added PNIPAM is

**Figure 3.6:** Fluorescence micrographs of hydrogel-HA mixtures using 10 wt% substitution of Rhodamine fluorescence labeled HA for (a) [HA] = 15 mg/ml without any microgel particles, (b) [HA] = 15 mg/ml and [PNIPAM] = 15 mg/ml, and (c) [HA] = 11 mg/ml and [PMSA] = 17 mg/ml.
Figure 3.7 (a) Linear viscoelastic spectra of measured elastic moduli, $G'$ (solid symbols) and viscous moduli, $G''$ (open symbols) as a function of radian frequency, $\omega$ at constant shear strain, $\gamma = 0.5\%$, and (b) non-linear viscous responses of measured $G''$ normalized by constant $\omega = 63$ rad/s as a function of shear strain, $\gamma$ for Rhodamine fluorescence labeled HA in aqueous suspensions of (i) [HA] = 10 mg/ml and added PNIPAM particles of [PNIPAM] = 15 mg/ml (black squares) and (ii) [HA]=15 mg/ml and [PNIPAM]=15 mg/ml (red circles). All samples were measured at constant temperature $T= 25 \, ^\circ\mathrm{C}$. 

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confirmed to be similar to that of plain HA systems as shown in Figure 3.7(a-b). For the simple fluorescence labeled HA solution, the fluorescence micrograph appears featureless as expected, suggesting a simple homogeneous solution. [84] Upon introducing non-fluorescent PNIPAM microgel particles, large clusters of size on the order of several microns emerge, indicating the aggregation regions of highly concentrated HA chains in complex networks are facilitated by added PNIPAM particles. As the number of PNIPAM particles added in HA aqueous solution is increased, these clusters grow in size and intertwine together, suggesting added PNIPAM particles work as the “crosslinker” in the HA matrix to cause the HA-PNIPAM complex formation. The complex formation strongly supports the observed rheological enhancement in HA-PNIPAM mixed suspension from plain HA solutions.

The aggregation of HA-PNIPAM mixtures is also confirmed in SEM images of dried HA-PNIPAM mixed samples in contrast to the individual dispersed PNIPAM particles without HA, shown in Figure 3.8(a-c). In previous studies, dried PNIPAM microgels form a crystal structure when dried and imaged using an SEM. [49] Also, due to the drying process, the microgels will shrink to ~50% of their size in solution. At low concentrations of microgels, [PNIPAM] = 15mg/ml, shown in Figure 3.8(a-b), dimers and trimers of particles are observed. At higher concentrations, [PNIPAM] = 30mg/ml, shown in Figure 3.8(c), much larger aggregates are observed. The presence of these aggregates can be attributed to attractive interactions between HA and the PNIPAM microgels. Furthermore, these particle aggregates are reminiscent of the aggregation of lubricin in SF, leading to speculation that the increased viscoelasticity results from a similar mechanism as the one present in SF. [72]
It is noted that the fluorescence micrographs and SEM images show the appearance of HA-PNIPAM aggregating microstructures only at relatively high HA concentration greater than 25mg/ml. Combined with the HA and PNIPAM concentration ranges at which the viscoelastic property of the mixed suspensions is comparable to or greater than that of human SF, the desired optimal concentration is narrowed to a range of $[\text{HA}] = 25$-30mg/ml and $[\text{PNIPAM}]= 15$-30mg/ml for effectively mimicking the structural and rheological characteristics of healthy SF. To examine the generality of using microgel particles as the HA crosslinker and rheological enhancer for HA suspension as artificial biolubricant, the microstructure of HA-PMSA mixtures is also characterized using 10 vol% fluorescence HA in aqueous suspensions. Similar fractal aggregates are observed by adding PMSA particles to plain HA suspension, despite the distinct LCST and UCST characteristics of PNIPAM and PMSA microgel particles at room temperature, respectively. It also further supports that the HA-aggregation is mainly due to the intermolecular hydrogen bonding between HA chains and microgel particles.

**Figure 3.8** Scanning electron micrographs of dried HA-PNIPAM mixed samples of (a-b) $[\text{HA}]=20$ mg/ml and $[\text{PNIPAM}]= 15$ mg/ml and (c) $[\text{HA}]=17.5$ mg/ml and $[\text{PNIPAM}]=30$ mg/ml. Micrograph (b) is the blow-out of (a) to show the aggregation of PNIPAM particles aggregation with the presence of HA polymers, which is in contrast to dispersed PNIPAM particles without added HA.
Last but not the least; the friction performance of HA-hydrogel mixtures is evaluated for their potential application as an artificial biolubricant by contact mode AFM. Due to the stronger grafting of PMSA particles to a pre-treated PGMA coating surface as well as their higher biocompatibility than PNIPAM particles, the local friction measurement of HA-PMSA mixed film is compared to the bare HA layer immersed in aqueous solutions. [83] The typical frictional micrographs are shown in Figure 3.9(a-b) and clearly indicate the high and low frictional regions by distinct color scales. By the aforementioned friction analysis, the frictional amplitude is determined against increased loading applied through AFM probe tips. Since the exact forces are difficult to determine from the AFM voltage signals, the exact friction coefficient, $\mu_{rel} = \partial F_N / \partial F_L$, cannot be easily calculated. Instead, as shown in Figure 3.9(c), a relative friction coefficient, $\mu_{rel}$ is calculated based on the difference between typical trace and retrace force signals as shown in the Inset of Figure 3.9(a-b), at varied normal loads. Figure 3.9(c) shows the measured $\mu_{rel}$ versus the normal load of the AFM tip. The presence of micron-sized hydrogels in the HA network thin films leads to increased surface roughness, resulting in high $\mu_{rel}$ at low loads for the PMSA-HA mixed regions compared to the HA regions. However, the focus of this study is the friction response at high loads, which is more comparable to the loads upon typical joint motions like flexion, and the high load asymptotic values of $\mu_{rel}$ for different polymeric films are compared in Figure 3.9(e). The highest $\mu_{rel}$ is observed with the bare PGMA thin film that is used as a universal, molecularly flat, and thin binding layer, with an average friction coefficient of $\mu_{rel}\approx0.013$. Importantly, the PMSA-added HA layer indeed exhibits the lowest friction
Figure 3.9: Representative AFM friction micrographs of grafted HA-PMSA mixtures to a PGMA-pretreated solid surface at normal loads of (a) 0 V and (b) 60 V over a scan area of 10 μm × 10 μm. The corresponding frictional amplitude of the trace (white lines) and retrace (yellow lines) are shown for the low friction PMSA regions (black circled areas) on the top panel and grafted PGMA (white circled areas) on the bottom panel. (c) Relative friction coefficient, $\mu_{rel}$, for PGMA, HA and PMSA determined from the plateau regime of normal load-dependent $\mu_{rel}$. 
coefficient of about $\mu_{\text{rel}} \approx 0.008$, in comparison to the HA layer of $\mu_{\text{rel}} \approx 0.008$. The low friction observed with PMSA-HA layer indeed is comparable to that reported for healthy SF, indicating the promise of HA-hydrogel mixtures as an artificial biolubricant. [67-70]

3.5 Conclusion

In summary, the applicability of the HA-microgel particle complexes is shown to make a biomimetic artificial biolubricant. These results have shown the aggregation of HA chains with added microgel particles in aqueous solutions, leading to the increased viscoelasticity of the mixed complex suspension from that of individual HA or microgel particles alone in suspensions of similar concentration. Similar rheology and aggregation structures are observed with both PNIPAM and PMSA microgel particles added to HA solutions, despite their distinct LSCT and UCST behaviors, respectively, suggesting the hydrogen bonding induced HA-microgel particle aggregation. By increasing the concentrations of low molecular weight HA chains and added microgel particles, the resulting rheological properties can be tuned to be comparable to, or higher than, that of healthy SF, showing great promises of using HA-microgel particle mixtures as a potential artificial biolubricant. Furthermore, the local frictional coefficient of HA-PMSA thin layers at optimal HA and PMSA concentration ranges are confirmed to be low within the reported low friction coefficient of healthy SF. Therefore, it is demonstrated that the HA-microgel particle aggregation via hydrogen bonds are designed as an artificial biolubricant to match the rheological and frictional properties of SF. In perspective, the binary mixture in this work can be further expanded into multi-component complex fluids.
to mimic the interfacial layer of SF in contact with articular cartilage and to achieve the full spectrum of the functionalities of SF.
CHAPTER 4:
GLASSY DYNAMICS OF DENSE MICROGEL SUSPENSIONS OF VARIABLE GLASS FRAGILITY

4.1 Introduction

While the previous chapter demonstrated the powerful versatility of microgels for biomedical design, this chapter delves into the use of microgels as tunable colloidal particles. Here, the glassy dynamics of microgel particles of variable elasticity is explored to elucidate the transition from fragile to strong glass-forming liquids. Through particle tracking algorithms, it demonstrated that controlling the elasticity of a microgel governs the glass fragility on the corresponding microgel suspension.

4.2 Background

As discussed in the introduction, the thermal behavior of dynamical quantities, such as viscosity, $\eta$, and relaxation time, $\tau$, of a molecular liquid approaching the glass transition temperature, $T_g$, has two limiting cases. [30-32] “Fragile” glass formers show a super-Arrhenius increase with temperature while “strong” glass formers show only an Arrhenius increase. Numerous experimental and theoretical studies have explored fragile and strong glass-forming behavior, but the underlying relaxation dynamics remains a
contentious topic. [29,30,87] Understanding the physical behavior leading to the differences between strong and fragile glass-forming liquids is beneficial for a broad range of applications involving amorphous materials, such as food and pharmaceutical sciences. [88-89]

Originally predicted in the Vogel-Fulcher-Tammann (VFT) law, dynamical quantities of glass-forming liquids increase exponentially with T approaching $T_g$. [30-32] Two popular theories support the VFT law: the Adam and Gibbs (AG) Theory and the Mode Couple Theory (MCT). AG theory proposes an entropic relaxation process, resulting from heterogeneous sub-regions that are characterized by cooperatively rearranging regions. [35] The growth of the length scale associated with these sub-regions implies a random first order transition from a liquid to a glass. On the other hand, MCT predicts the glass transition is a localized process governed by the structural arrest of particles escaping the “cages” generated by their nearest neighbors. MCT describes particle relaxation in two regimes: the $\alpha$ and $\beta$ regimes. The $\alpha$ regime occurs at long timescales describing the movement of particles away from the cages. The $\beta$ regime occurs at small time-scales describing the ballistic particle motion inside their cages.

While these theories agree with numerous experimental and computational results, little insight is offered into the underlying physics responsible for the differences in glass fragility. It is theorized that the particle motion of fragile glass-forming systems is a process dominated by relaxation dynamics. [90-91] Once the system reaches a critical temperature, the particles become jammed, leading to the abrupt increase in $\eta$ and $\tau$. For strong glass-forming systems, the particle motion is vibration dominated. [90-91] This
results in a strong correlated motion between particles preventing the onset of a jammed state.

Although a direct visual study of particle motion in molecular glasses is impossible, particle dynamics of colloidal suspensions is directly observable and quantifiable using several microscope and particle tracking techniques. [92-94] Dense colloidal suspensions exhibit similar glassy behavior to molecular liquids with the dynamic properties dependent on the colloidal concentration rather than the temperature. Extensive studies on “hard-sphere” colloidal suspensions have revealed model fragile glassy behavior. [23,92-94] These suspensions exhibit the onset of a “super-cooled” state at a volume fraction, $\phi$, of 0.53 characterized by slowed-down motion. The glass transition volume fraction, $\phi_g$, is observed at $\phi=0.58$, noted by arrested motion in a jammed state. [23,92] Recent studies on suspensions of soft, deformable colloids revealed strong glass-forming behavior, characterized by a delayed onset of a jammed state. [23,95] However, only a few recent studies have reported direct visual evidence of this behavior. [96-97] Furthermore, most studies do not account for the shrinkage of deformable microgels at high concentrations: this leads to overestimates of the actual volume fraction, thus a lack of knowledge of its dependence of the super-cooled and glassy regimes.

In this chapter, the glassy dynamics of one microgel system of varied stiffness is explored to elucidate the transition from strong to fragile glass-forming behavior where the inter-particle dynamics will be directly observed via confocal microscopy at single particle resolution. The mobility and distribution of the microgels are analyzed using image processing and particle tracking algorithms. Here, control over the glass fragility is shown by tuning the microgel elasticity during the synthesis. For all particle elasticity
observed, there is evidence of the caging of particles by their nearest neighbors at high concentration. As expected, this results in slowed-down and arrested motion. Furthermore, the scaling of the relaxation times of the particles escaping their cages compares well with behavior predicted by the AG theory.

4.3 Experimental

4.3.1 Materials

Poly(N-isopropylacrylamide) (PNIPAM) microgels are synthesized using the method of free radical polymerization described in Chapter 2. [49] Methylene-bis-acrylamide (BIS) is used as the crosslinker and the microgel elasticity is controlled by varying the crosslinker ratio, \( CL = \frac{[\text{BIS}]}{[\text{BIS}]+[\text{NIPAM}]} \), where [BIS] and [NIPAM] are the mass concentrations of BIS and NIPAM, respectively, during the synthesis. Five different crosslinker (CL) ratios are examined in order to observe a range of particle deformability. These values are CL=0.9%, 1.6%, 2.2%, 3.3%, and 6.6%. The sample is concentrated into a dense suspension using a Rotovap R-210 at 60°C and diluted with deionized water until the desired concentration is achieved.

4.3.2 Image Acquisition

Suspensions of microgels are imaged in three-dimensions via confocal microscopy, typically 30μm x 30μm x 12μm image box in the x-y-z directions, over a long time frame, up to ~14 hours for dense samples. A 3D image is formed by acquiring succinct 2D images in the x-y plane over short intervals in the z-direction, creating a “z-stack”. Temporal and spatial data regarding each individual microgel is measured via 3D particle
centroiding algorithms. Originally developed by Crocker and Weeks using the image analysis code Interactive Data Language (IDL), these algorithms determine the center of a particle with an accuracy of 0.05 μm in the x-y plan and 0.2 μm in the z direction. [98] Many studies have used this code as the initial step of measuring particle dynamics in glassy suspensions. [93-94]

4.3.3 Particle Tracking via Image Analysis

These algorithms work in a two-step process. First, the raw image is processed so the particles are clearly definable to be tagged. A band-pass filter is applied to the image, which eliminates any low and high frequency contributions. This routine convolves the image using a Mexican hat wavelet transform in three steps: the x-direction, y-direction, and the z-direction. [98] Successive convolutions often introduce errors in the processed images, e.g. pixel biasing and clipping of particles, but these effects should be considered negligible due to the large number of particles in these systems. The input to this algorithm requires definitions for a “low pass” filter and a “high pass” filter. The low pass filter eliminates the single pixel noise in the raw image while high pass filter is typically the diameter of particles in pixels. [98] Due to difference in resolution between the x-y plane and the z-direction, the high pass filter must be measured for the sample prior to inputting it into the algorithm. This is accomplished using additional algorithms which reconstruct the z-stack to view the image in the x-z and y-z planes. The pixel diameter is then measurable in all three directions. Due to poor resolution in z-direction, the high pass filter is typically larger than it is for the x-y plane.
The second step of this process is to extract the coordinates of all the particles in each 3D image. The first part of this process involves finding the local maximum in pixel brightness of the convolved image. Using a spherical mask, this routine approximates the centroids of the particle candidates at location (x,y,z). This approximation is refined by calculating the shifts in the center \((f_x, f_y, f_z)\)

\[
\begin{pmatrix}
  f_x \\
  f_y \\
  f_z
\end{pmatrix} = \frac{1}{M} \sum_{i} \sum_{j} \sum_{k} \sum_{i^2 + j^2 + k^2 \leq w^2} l(i, j, k) (a + i, b + j, c + k)
\]  

(4.1)

Here, M is the integrated intensity and w is the mask radius. The particles’ positions are refined by shifting \((f_x, f_y, f_z)\) to \((f_x + a, f_y + b, f_z + c)\). These algorithms were written for analyzing dilute systems and pixel biasing has been observed in dense systems. [98] To avoid the pixel biasing, the size of the mask is chosen to be a value slightly smaller than the diameter of the particles. Choosing the best size of mask requires a trial-and-error method. Pixel biasing is tested by creating a histogram of the fractional pixel values. [98] If the histogram exhibits large maxima, the image processing is plagued by pixel biasing and the input parameters should be refined until no maximum is observed.

Once there is confidence that each particle’s location is properly tagged in the 3D stack, it is now the aim to link the particle’s position from frame to frame in order to develop trajectories. To perform this analysis, first a cost function is established to calculate the mean square frame-to-frame displacement (MSFD) of the particles.

\[
MSFD = \sum_{j=1}^{N} (r_{i+n,j} - r_{i,j})^2
\]  

(4.2)

Here, \(r\) is the location of the particle and \(n\) is the frame number. Minimization of this cost function is the aim to determine the most accurate particle trajectories, where the particle motion does not exceed the inter-particle spacing within each frame. [98] The tracking
algorithm works by defining a tracking radius, $R_T$, and finding all the particles which have moved within $R_T$ which minimalizes MSFD. This function examines all the particles in a given frame and determines all possible locations of the particles in the subsequent frame that have moved less than $R_T$. This method leads to a degree of error as when a particle motion exceeds $R_T$, it may be misidentified or assigned a new tracking ID. Therefore, it is important that $R_T$ accurately characterizes the average distance a particle travels between frames. To test the accuracy of the chosen $R_T$, a histogram is analyzed of particle displacements with a lag time of $\tau = 1$ in the x, y, and z-directions. If the maximum displacements are large, e.g. greater than $O(1)$, then it is likely that particles are misidentified. In that case, a new value of $R_T$ is chosen for the tracking algorithms and the analysis is repeated. As with algorithms which tag the particle coordinates, the tracking procedures require a degree of trial-and-error. Once the trajectories of each particle are known, many dynamic properties about the suspension are measureable, e.g. the mean-squared displacement.

4.4 Results & Discussion

The microgel stiffness is first observed through the size change of the hydrodynamic diameter, $d_H$, across the lower critical solution temperature, LCST, of PNIPAM, shown in Chapter 2 in Figure 2.2. In water, the swollen PNIPAM particles collapse as the temperature, T, increases from 25°C to 40°C. This collapse occurs as T increases past the LCST of PNIPAM, ~32°C, indicating an LCST range of T=26 –39 °C. This behavior is consistent with previous studies on PNIPAM microgels. [49,58] As expected, decreasing the CL ratio results in a greater size change. It should be projected that smaller CL ratios
will lead to more deformable particles. Due to the compressibility of softer particles, the hydrodynamic diameter does not accurately represent the size of the microgel at high concentrations. This is confirmed by observing the average center-to-center separation, \( R_0 \), of the microgels, which is determined from the first peak of the pair correlation function,

\[
\int_0^\infty \rho g(r) 4 \pi r^2 dr \approx N, \tag{4.1}
\]

where \( r \) is the radial distance from center of the particle, \( N \) is the number of particles in the sample, and \( \rho \) is the number density of particles. For \( CL = 0.9\% \), shown in Figure

**Figure 4.1:** The pair correlation function, \( g(r) \), of dense PNIPAM microgel suspensions plotted against normalized radial distance, \( r/d_H \), for (a) \( CL = 0.9\%: \phi=0.46 \) (black), \( \phi=0.69 \) (red), \( \phi=0.71 \) (green), and \( \phi=0.95 \) (blue), (b) \( CL = 2.2\%: \phi=0.38 \) (black), \( \phi=0.47 \) (red), \( \phi=0.50 \) (green), \( \phi=0.59 \) (blue), \( \phi=0.67 \) (cyan), and \( \phi=0.79 \) (magenta), and (c) \( CL=6.6\%: \phi=0.38 \) (black), \( \phi=0.49 \) (red), \( \phi=0.51 \) (green), \( \phi=0.56 \) (blue) and \( \phi=0.58 \) (cyan)
4.1(a), $R_0$ is characterized by a gradual shift away from $d_H$, suggesting that these particles are very susceptible to changes in $\phi$. Increasing the CL ratio to 2.2%, the shift of the primary peak reaches a minimum value at large concentrations, shown in Figure 4.1(b). This suggests that the microgel has greater mechanical strength, signifying a transition to hard-sphere behavior. For the hard-sphere colloids, the particle diameter should be independent of concentration. This is nearly the case for CL=6.6%, shown in Figure 4.1(c), where there is only a minimal change in the initial peak as the concentration is increased, suggesting $R_0$ is relatively unaffected by changes in concentration.

Based on the microgel deformability, an adaptive model is required to determine the actual size and concentration of the microgels. When microgels come into contact, there is effective overlapping with a minimum distance between two microgels of $R_0$ and the maximum separation distance of $d_H$. The effective radius, $r_{eff}$, of a microgel is between these two values and can be defined by the root mean square of the two radii,

$$r_{eff} = \frac{1}{2} \sqrt{R_0 d_H}.$$  \hfill (4.2)

Now, $\phi$ is redefined as

$$\phi = \rho \cdot \left( \frac{4}{3} \pi r_{eff}^3 \right).$$  \hfill (4.3)

This may slightly overestimate the actual volume fraction of suspension of very soft particles, but should be considered more accurate than previous definitions. Furthermore, using $r_{eff}$, the deformability of these microgels is defined by fitting the pair potential, $\beta V(r) = -\ln(g(r))$, where $\beta^{-1} = k_B$, with a Hertzian model, [95]

$$\beta V(r) = \frac{4}{15} E(1 - \frac{r}{\sigma})^{5/2}, \ r \leq \sigma.$$  \hfill (4.4a)

$$\beta V(r) = 0, \ r > \sigma$$  \hfill (4.4b)
For this system, $\sigma = 2r_{\text{eff}}$ is the particle diameter and $4E/15$ is a dimensionless temperature which quantifies the particle stiffness. This model is only valid for single contact between two particles and, therefore, is only applicable for an intermediate range of concentrations. The relationship, $E^* = E k_B T / V_C$, is used to estimate the elastic modulus of the microgel where $V_C$ is the volume of the contact area. The dependency of $E^*$ on CL is shown in Figure 4.2, which shows a super-Arrhenius increase in particle elasticity. Interestingly, for the three smallest CL ratios, $E^*$ grows gradually in a nearly linear fashion then shows exponential growth for the largest CL ratios. This suggests a critical point above CL=2.2% where the CL ratio has strong control of the microgel elasticity. Based on this behavior, it is expected that the low elasticity limit for these microgels is achieved at the smallest CL ratios.

**Figure 4.2:** The elastic constant ($E^*$) plotted against the CL ratio as determine from Hertzian model presented in Equations 4.4a&b.
Based on the deformability of these PNIPAM microgels, it is expected that decreasing $E^*$ from $2.6 \times 10^4 \text{Pa}$ to $166 \text{Pa}$ will capture the transition from fragile to strong glass-forming behavior in dense suspensions. Visual evidence of a disordered particle distribution is observed in the confocal images, shown in Figure 4.3 for $E^* = 166 \text{Pa}$ and $E^* = 2.6 \times 10^4 \text{Pa}$. At the range of concentration observed for both microgel systems, the particles remain disordered and there is no evidence of a crystalline state. The samples are confirmed to be in a disordered state using Voronoi tessellation, shown in Figure 4.4. A Voronoi tessellation defines the volume region, Voronoi volume, of each individual particle in a space defined by their nearest neighbors. [28] For a perfect crystalline material, each particle will be equally spaced from their nearest neighbors and a very

![Figure 4.3: Sample images of the Confocal micrographs for (i) $E^* = 2.6 \times 10^4 \text{Pa}$: (a) $\phi=0.38$, (b) $\phi=0.50$, and (c) $\phi=0.58$ and (ii) $E^* = 166 \text{Pa}$: (d) $\phi=47$, (e) $\phi=0.75$, and (f) $\phi=0.95$. Scale bar is $5 \mu\text{m}$.

Based on the deformability of these PNIPAM microgels, it is expected that decreasing $E^*$ from $2.6 \times 10^4 \text{Pa}$ to $166 \text{Pa}$ will capture the transition from fragile to strong glass-forming behavior in dense suspensions. Visual evidence of a disordered particle distribution is observed in the confocal images, shown in Figure 4.3 for $E^* = 166 \text{Pa}$ and $E^* = 2.6 \times 10^4 \text{Pa}$. At the range of concentration observed for both microgel systems, the particles remain disordered and there is no evidence of a crystalline state. The samples are confirmed to be in a disordered state using Voronoi tessellation, shown in Figure 4.4. A Voronoi tessellation defines the volume region, Voronoi volume, of each individual particle in a space defined by their nearest neighbors. [28] For a perfect crystalline material, each particle will be equally spaced from their nearest neighbors and a very
A narrow peak would be expected for the Voronoi volume of the particles. As the particles become more disordered, these peaks become broader. For all three microgel systems observed in Figure 4.4, there is a broad distribution of the Voronoi volumes suggesting a disordered state is observed for all concentrations. Also, the shift of the peak to smaller volumes at higher concentrations confirms that deformable microgels are shrinking at high concentrations.

To start characterizing the glassy dynamics of the microgel suspensions, the concentration-dependent mobility of the particles is assessed to determine when the particles’ motion becomes arrested. The mobility of the particles is first measured using the mean squared displacement (MSD) of the particles as function of the observation time.
\[< \Delta x^2 > = \frac{1}{N} \sum_{j=1}^{N} [x_j(0) - x_j(\tau)]^2 \]  
(4.5)

This function is averaged over all particles in the scanning area when \( \tau \) is the initial lag time along the x coordinate. [99-100] Due to differences in the size of the microgels at different CL ratios, \( \tau \) is normalized by a scaling factor \( k \), which is the ratio diffusion time of particles in dilute suspensions and the diffusion time, \( \tau_D \), of the stiffest particle calculated using the Einstein-Stokes equation,

\[ k = \frac{\tau_D}{\tau_{D,CL=6.6\%}} \]  
(4.6)

For the smallest elasticity, \( E^* = 166 \text{ Pa} \), Figure 4.5(a), from \( \phi = 0.49 \) to \( \phi = 0.61 \), there is very minimal change in mobility, which is contrary to what would be expected for a hard-sphere fragile glass former. Due to the deformability of the particles, increasing \( \phi \) leads to
a decrease in particle size, rather than arrested particle motion. Slowing of particle motion is only measured at high volume fractions, $\phi=0.66$. Arrested motion is exhibited at $\phi=0.74$ and $\phi=0.82$ for long timescales where the nearest neighbors have caged the particles in a jammed state. At the intermediate elasticity, $E^*=179$ Pa, shown in Figure 4.5(b), there is a drop in mobility from $\phi=0.38$ to $\phi=0.61$, characteristic to fragile glass. Yet, the onset of an arrested state is not observed until high concentrations, such as $\phi=0.79$, which is indicative of strong glass-forming behavior. This suggests that these microgels form an intermediate state between a fragile or strong glass former. At the highest elasticity, $E^*=2.6\times10^4$ Pa, Figure 4.5(c), there is a dramatic decrease in mobility at the concentration, characteristic of hard-sphere behavior. Particle motion is rather diffuse at $\phi=0.38$ and becomes arrested in an apparent jammed state at $\phi=0.58$.

The MSD results indicate that the concentration at which particles become caged is dependent upon the microgel elasticity. The structural relaxation of the particles from these cages is probed to further to elucidate the effect of microgel deformability on the glassy dynamics of these dense suspensions by measuring the overlap order parameter,

$$q_s(\tau) = \frac{1}{N} \sum_{i=1}^N w(|r_i(\tau) - r_i(0)|);$$  \hspace{1cm} (4.7a)

$$w = 1(0) \text{ if } |r_i(\tau) - r_i(0)| < (>)a.$$  \hspace{1cm} (4.7b)

This quantifies the number of “overlapping” particles separated by a distance, $a$, over the observation time; thus providing a method to measure the localization of particles as a function of lag time. Here, $a$ is taken as $0.5d$ to distinguish between localized and delocalized particles. \[93\] At $E^*=166$ Pa, shown in Figure 4.6(a), there is a gradual evolution of slow dynamics spanning a wide range of $\phi$. Up to $\phi=0.61$, the particles will readily separate from their nearest neighbors, shown by the exponential decay of $q_s(\tau)$. At
$\phi = 0.66$, evidence of trapped particles is observed as $q_s(\tau)$ remains near unity for $\sim 2000$ s.
 Increasing $\phi$ results in progressively slower particle dynamics, as the time scales at which particles escape their cages increases dramatically. At the intermediate $E^*$, shown in Figure 4.6(b), the caging of microgels occurs over a smaller range of $\phi$, similar to the onset of arrested particle motion observed in the MSD. An exponential decay of $q_s(\tau)$ is observed for $\phi = 0.38$ and $\phi = 0.52$ and the onset of caging observed for $\phi = 0.61$. At the highest concentrations, $\phi = 0.68$ and $\phi = 0.79$, evidence of a jammed state is present as the particles will remain trapped by their nearest neighbors for the duration of the observation period. For $E^* = 2.6 \times 10^4$ Pa, shown in Figure 4.6(c), particles become localized over short range of $\phi$. At $\phi = 0.38$, there is little evidence of jamming as particles rapidly separate from their neighbors. Indications of caging is present at $\phi = 0.48$, and there is significant evidence of jamming at highest concentrations.
From the analysis of $q_s(\tau)$, it is possible to develop a phase diagram to further show the effect of microgel deformability on the glassy dynamics of dense suspensions, shown in Figure 4.7. A “liquid” phase is characterized by an exponential decay of $q_s(\tau)$, as microgels are able to readily move away from their neighbors. A “glass” phase occurs when $q_s(\tau)$ decays to a value of 1/e at times greater than a characteristic timescale for a glassy state, $\tau_g$. [93] For this work, $\tau_g$ is chosen to be 100,000s since it recovers a glass transition volume fraction, $\phi_g$, of 0.58, for $E^* = 2.6 \times 10^4$Pa which was previously shown as the $\phi_g$ for hard-sphere PNIPAM microgel suspensions. [23] This results in a third, intermediate phase, distinctive of the “super-cooled” phase, as the microgels experience
significant slow dynamics yet a glassy state is not observed. For the two highest elasticity constants, 436Pa and 2.6x10^4 Pa, this phase diagram shows behavior expected for hard-sphere colloids. The glass transition remains constant at $\phi=0.58$ and there is a narrow range of concentrations for the super-cooled regime. This abrupt transition from the liquid phase to glass phase suggests that these microgels behave like strong glass formers.

Decreasing the elasticity to $E^*=179$Pa results in an extended super-cooled regime and a delayed onset of the glassy state. The concentration range of the super-cooled phase increases as the elasticity is lowered to $E^*=169$Pa and $E^*=166$Pa. These results show a much more gradual transition from a liquid-state to a glassy-state signifying a strengthening of the glass formers. The phase transition from liquid to super-cooled regimes is apparently independent of the fragility of the glass as the onset of the super-cooled regime remains relatively constant at all values of $E^*$. This transition likely coincides with the volume fraction at which the microgels come into contact. For hard-sphere particles, a small increase from this volume fraction would lead to a jammed state. For deformable particles, the pressure induced by the neighboring particles causes the microgels to shrink and results in extended volume fractions at which the particles remain mobile.

Fitting $q_s(\tau)$ using the Kohlrausch-Williams-Watts (KWW) formula extracts relevant timescales for single-particle rearrangements. [93]

$$q_s(\tau) = A \exp\left(-\left(\frac{\tau}{\tau_\alpha}\right)^\beta\right)$$  \hspace{1cm} (4.8)

Here, $\tau_\alpha$ is the timescale related to the alpha relaxation process and $A$ and $\beta$ are free floating parameters. These timescales are normalized by the scaling factor $k$, as described
previously, and shown in Figure 4.8(a). Using an Adams-Gibbs (A&G) relationship, $\tau_\alpha$ is fitted as a function of $\phi$, 
\[
\tau_\alpha = C \cdot \exp\left(\frac{B}{\phi}\right),
\]
(4.9)
where $B$ and $C$ are free floating parameters. The $\phi_g$ for each elasticity is determined using Equation 4.9 by interpolating $\phi$ at $\tau_\alpha = \tau_g$. An examination of the relationship between $k^*\tau_\alpha$ versus $\phi/\phi_g$, shown in Figure 4.8(b), illustrates that the transition between a strong to a fragile glass-forming system occurs over a small range of elasticity. For the two stiffest microgels, there is a sharp increase in $\tau_\alpha$ as the glass transition is approached, which corresponds to the behavior of fragile glass. For $E^*=179\text{Pa}$, $E^*=169\text{Pa}$ and $E^*=166\text{Pa}$, this increase becomes more gradual, more characteristic of a strong glass-forming system.

The A&G model shows good agreement with the measured $\tau_\alpha$ for the entire spectrum of $\phi$ analyzed for each stiffness value. This agrees with previous studies that the relaxation of suspensions of hard-sphere particles is an entropic process which occurs through collective motion. [93] However, it is also interesting to note that this model predicts the relaxation of particles in strong glass-forming suspensions and is governed by a similar process. The validity of this model is further tested by calculating the fragility of the glass using the following definition of the fragility index,
\[
m = \left[\frac{d[\ln(\tau_\alpha)]}{d(\phi/\phi_g)}\right]_{\phi=\phi_g} = -\frac{B}{\phi_g},
\]
(4.10)
which measures the slope of the A&G fitting at the glass transition, shown in the inset of Figure 4.9. The two stiffest microgels have the highest index value, $m\approx10$, and there is a sharp transition to the two softest microgels, which have smaller index values, $m\approx4$. This sharp transition from fragile to strong glass-forming behavior shows strong agreement
Figure 4.8: (a) The scaled $\alpha$-relaxation time, $\tau_\alpha$, plotted against volume fraction, $\phi$, for $E^* = 166\text{Pa}$ (black squares), $E^* = 169\text{Pa}$ (red circles), $E^* = 179\text{Pa}$ (green triangles), $E^* = 436\text{Pa}$ (inverted blue triangles), and $E^* = 2.6 \times 10^4\text{Pa}$ (cyan diamonds). (b) Same as (a), plotted against the normalized volume fraction by the glass transition, $\phi_g$. Solid line representing the fitting of the A&G model.
with predicted behavior shown in the phase diagram. Furthermore, the trend of this index suggests that an intermediate regime between fragile and strong glass formers only exists for a very distinct range of microgel stiffness. Otherwise, it is expected that soft, deformable particles will behave as strong glass formers and stiff, hard sphere particles will exhibit hard-sphere behavior.

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$$m = \frac{d[\ln(\tau_\alpha)]}{d(\phi/\phi_g)} \bigg|_{\phi=\phi_g} = -\frac{B}{\phi_g}, \quad (4.10)$$

which measures the slope of the A&G fitting at the glass transition, shown in the inset of Figure 4.9. The two stiffest microgels have the highest index value, $m \approx 10$, and there is a sharp transition to the two softest microgels, which have smaller index values, $m \approx 4$. This sharp transition from fragile to strong glass-forming behavior shows strong agreement with predicted behavior shown in the phase diagram. Furthermore, the trend of this index suggests that an intermediate regime between fragile and strong glass formers only exists for a very distinct range of microgel stiffness. Otherwise, it is expected that soft, deformable particles will behave as strong glass formers and stiff, hard-sphere particles will exhibit hard-sphere behavior.
4.5 Conclusions

In summary, the transition from strong to fragile glass-forming behavior for a single microgel system of varying stiffness is shown through a direct microscopic study. Particles with the highest elasticity exhibited the fragile glass-forming behavior expected for a hard-sphere suspension. Strong behavior was observed for softer particles, characterized by a delayed onset of the glass transition and an extended super-cooled regime. This phenomenon results from the shrinking of deformable particles at high concentrations, which leads to extended particle mobility rather than a jammed state. The relaxation of these suspensions is described well by an A&G relationship, which
accurately predicts the fragility and $\phi_g$ of the suspension. The next chapter will explore how dynamic heterogeneities, which are predicted by A&G’s model, affect the relaxation process of strong and fragile glass-forming PNIPAM suspensions.
5.1 Introduction

With confirmation that controlling microgel elasticity changes the fragility of the glass-forming suspension, it is now of interest to analyze the heterogeneous dynamics involved in strong and fragile glass-forming liquids. The microgel suspensions from the previous chapter will be further analyzed via particle tracking algorithms to elucidate length scales associated with dynamic heterogeneity and to observe variations in string-like motion. It is also the aim to observe how the CRRs vary as the material transitions from strong to fragile glass-forming behavior.

5.2 Background

As specified in Chapter 1, super-cooled liquids are spatially heterogeneous, as characterized by the presence of CRRs. Links between glass fragility and dynamic heterogeneity are established for molecular glasses. [101-104] Initial studies inferred spatial heterogeneity through evidence of heterogeneity in timescales. Therefore, one measure of dynamic heterogeneity is the stretching exponent, β, of the Kohlrausch-
Williams-Watts (KWW) formula, defined as $\phi(t) = e^{-(t/\tau_{KW})^\beta}$ for analysis of light scattering measurements. [102] As $\beta$ decreases, there is greater heterogeneity in the timescales for the glass-forming liquid. It was shown that $\beta$ is the smallest for fragile glass formers and increased with decreasing fragility. [102] These results concluded that fragile glass formers are more spatially heterogeneous than strong glass formers. Recent studies also demonstrated the importance of dynamic facilitation for particle motion in strong glass-forming liquids, which prevents a jammed state. [105-106] This is characterized by the presence of long string-like particle motion.

Many experimental studies have turned to colloidal suspensions to analyze dynamic heterogeneity. [101,107-108] However, these experiments have typically focused on model hard-sphere particles, thus have deduced evidence of dynamic heterogeneity for fragile glass-forming liquids. Typical hard-sphere colloidal suspensions with concentrations close to $\phi_g$ exhibit strong heterogeneous dynamics characterized by clusters of mobile and immobile particles. In a simulation study of polydisperse colloidal suspensions, clusters of immobile particles grew as the concentration increased through the super-cooled phases. [101] The structural order in these immobile clusters led to the presence of dynamic heterogeneity, leading to the conclusion that the origin of dynamic heterogeneity is thermodynamic rather than kinetic.

Experimental studies employing dynamic light scattering techniques to analyze dynamic heterogeneity in super-cooled colloidal suspensions quantify dynamic heterogeneity with the three-point dynamic susceptibility function, defined as $\chi_\phi(q, t) \equiv \partial F_S(q, t) / \partial \phi$. [107] Here, $F_S$ is the intermediate scattering function defined as $F_S(q, t) = \frac{1}{N} < \rho_q(t)\rho_{-q}(0) >$, where $\rho_q$ is the Fourier component of the density and $N$ is the
number of particles. This powerful function relates structural relaxation to fluctuations in the particle density. Dynamic heterogeneity is inferred from $F_S$ by calculating $\chi_\phi$, which represents the number of particles that have correlated dynamics, a signature for the presence of CRRs. These studies show an exponential increase in correlated particles with increasing volume fractions through the super-cooled regime, concluding that the growth of correlated particles is directly tied to the mobile and immobile clusters of particles. [107] Relating CRRs to fluctuations in particle density provided further evidence that dynamic heterogeneity is entropic in nature.

While dynamic light scattering is a robust measure of dynamic heterogeneity, it does not provide direct visual evidence of the mobile and immobile clusters. One method often employed to directly observe dynamic heterogeneity in colloidal glasses is to use confocal microscopes to observe inter-particle dynamics of fluorescent colloids in three dimensions. Due to the size of the colloids, the individual particle motions are on timescales that are observable to the microscope camera. Previous studies using a direct microscopic study demonstrated that correlated particle motion is observed over distances of 3-4 particle diameters. [108] The largest degree of correlation is observed at timescales associated with cage rearrangements. Furthermore, the correlated particle motion grows precipitously as the sample approaches the glass transition.

In this chapter, the analysis of dynamic heterogeneity using fluorescent colloidal suspensions is expanded to include particles of tunable elasticity to expound the differences for strong and fragile glass-forming suspensions. Individual particle mobility will be analyzed using confocal image processing and particle tracking techniques. It is shown here that individual particle mobility is spatially heterogeneous and governed by
caging from nearest neighbors. This leads to the presence of mobile and immobile clusters. As expected, mobile clusters are characterized by CRRs in the form of string-like motion. The growth of length scales and the size of the strings associated with dynamic heterogeneity are both shown to be dependent on the glass fragility.

5.3 Experimental

The same aqueous suspensions of PNIPAM microgel particles analyzed in the previous chapter are used again for this analysis with an average \( d_H \) of 1.66 \( \mu \)m \( \pm 0.25 \) \( \mu \)m and CL ratios of 0.9\%, 1.6\%, 2.2\%, 3.3\%, and 6.6\%. The elasticity, \( E^* \), values for these microgels are 166 Pa, 169 Pa, 179 Pa, 436 Pa, and \( 2.6 \times 10^4 \) Pa as calculated from the Hertzian model. Therefore, the relaxation dynamics and fragility for each microgel system is already established and well known. Since dynamic heterogeneity is most prominent in the super-cooled phase, concentrations associated with that phase will be analyzed for this study. Time series confocal images (Zeiss LSM 5 Pascal, 100x objective, NA = 1.4) are analyzed via IDL particle tracking algorithms which were introduced in the previous chapter. While the previous algorithms probed the assembled average motion and relaxation of the tracked particles, it is now the goal to target individual particle motions.

5.4 Results & Discussion

The Adam & Gibbs relationship proposes that the onset of the super-cooled regime of a glass-forming liquid is induced by caging of the individual particles. [25-28,34] The effect of caging is observed by measuring the “cage rattling” of the particle, which are the
short, fast movements of the particle while still in the ballistic time range. One measure of the cage rattling process is the Debye-Waller (DW) factor, \(<u^2>\), which is the mean-squared displacement, \(<r^2>\), at a fixed time interval. [109] By choosing a short time interval, the DW factor can characterize the distance a particle moves inside its cage. Here, \(<u^2>\) is defined as \(<r^2(\tau = \tau_1)>\), where \(\tau_1\) is the time between subsequent image frames. This ensures that all concentrations of colloids will be in the ballistic regime as demonstrated by the overlap order parameter in the previous chapter. The comparison of \(<u^2>\) versus \(\phi\) is shown in Figure 5.1(a) for each microgel system. It is evident from this plot that there is a step transition from a “fast” rattling process, with particle motion on the order of 0.1\(\mu m^2\), to a “slow” rattling process, with particle motion on the order of 0.01\(\mu m^2\). It is proposed here the strong decrease in the rattling process is induced by caging of the particles, and the volume fraction at this transition is defined as the caging volume fraction, \(\phi_c\), shown in Figure 5.2. Normalizing the cage rattling process by scaling \(\phi\) by \(\phi_c\) and \(<u^2>\) by \(r_{\text{eff}}^2\), shown in Figure 5.1(b), creates a master curve of the caging process for a glass liquid showing the universality of the caging process for all particle elasticity. This provides further evidence to suggest that the Adam & Gibbs model is applicable for all glass fragilities.

While the caging process universally governs the slowing down of particle mobility in glassy suspensions, Figure 5.2 demonstrates that \(\phi_c\) is dependent on the particle elasticity, and therefore directly related to the glass fragility. Moreover, returning to the phase diagram, shown in Figure 4.7, it is now apparent that the dependence of \(\phi_c\) on \(E^*\)
Figure 5.1: (a) The average Debye-Waller factor, $\langle u^2 \rangle$, scaled by the effective microgel radius, $r_{\text{eff}}$, versus volume fraction, $\phi$, for $E^* = 166\, \text{Pa}$ (black squares), $E^* = 169\, \text{Pa}$ (red circles), $E^* = 179\, \text{Pa}$ (green triangles), $E^* = 436\, \text{Pa}$ (inverted blue triangles), and $E^* = 2.6 \times 10^4\, \text{Pa}$ (cyan diamonds). (b) Same as (a.) with $\langle u^2 \rangle / r_{\text{eff}}$ now versus $\phi$ scaled by the caging volume fraction, $\phi_C$. 
corresponds to the $\phi$ at which the liquid to super-cooled phase transition occurs, as determined from the analysis of the overlap order parameter. This alludes to a connection between the caging process and the onset of the super-cooled phase in colloidal glasses. Therefore, caging leads to the slowing down dynamics in glasses that precedes the onset of the glass transition. In strong glass-forming liquids, the caging process is extended over a larger range of $\phi$ whereas that regime is much smaller for fragile glass-formers.

While the Debye-Waller analysis provides further evidence to suggest the caging of particles induces the super-cooled phase, the ensemble average does not offer insight into the homogeneity of the cage rattling process. To elucidate the evolution of the caging process, the Debye-Waller factor, $u^2$, is mapped for the individual particles shown in

**Figure 5.2** The caging volume fraction, $\phi_C$, versus microgel elasticity, $E^*$, as defined from the analysis of the Debye-Waller factor.
Figure 5.3 for microgel systems with elasticity of $E^* = 166 \text{ Pa, } 179 \text{ Pa, and } 2.4 \times 10^4 \text{ Pa}$.

As expected, particles display faster cage rattling below $\phi_c$ and that motion is significantly suppressed above $\phi_c$. Interestingly, the clusters of mobile and immobile particles form with a high degree of inhomogeneity below and above $\phi_c$ for the entire range of particle elasticity. Both regimes display clusters of particles with high mobility and with low mobility. This may suggest that inhomogeneity originates in the liquid phase and propagates in the super-cooled and glass phases, which supports previous reports that dynamic heterogeneity is derived from fluctuations in particle density. [25]

This agrees with studies that suggest dynamic heterogeneity is an entropic event. [101] If highly mobile particles exist in low density regimes and low mobile particles exist in high

![Figure 5.3: The Debye-Waller factor, $u^2$, for each individual particle for microgel suspensions above the caging volume fraction, $\phi_C$, (a-c) and below $\phi_C$ (d-f) for microgel elasticity $E^* = 166 \text{Pa}$ (a) $\phi = 0.56$ & (d) $\phi = 0.76$, $E^* = 179\text{Pa}$ (b) $\phi = 0.52$ & (e) $\phi = 0.68$, and $E^* = 2.6 \times 10^4 \text{Pa}$ (c) $\phi = 0.49$ & (f) $\phi = 0.76$. Colors correspond to the pallet legend on the right of the color maps.](image-url)
density regimes, the mobile clusters would become jammed into an immobile state as the sample is quenched to high concentrations. The clusters of highly mobile particles would be left in low density pockets resulting in mobile clusters.

The probability distribution of the DW factor, \( P(u^2) \), which corresponds to the previous mobility maps, shown in Figure 5.4, show a broad range of particle mobility above and below \( \phi_c \). Clearly, the peak probability represents the average particle mobility with a sharp decrease in the peak location above \( \phi_c \). However, the distribution of mobility is rather broad with a long tail of mobile particles. Based on the map of mobile particles and the corresponding probability distributions, it is evident that mobile and immobile clusters are predominant in the microgel suspensions for a wide range of \( \phi \).

Figure 5.4: The probability distribution of The Debye-Waller factor, \( P(u^2) \), for each individual particle for microgel suspensions above the caging volume fraction, \( \phi_c \), (a-c) and below \( \phi_c \) (d-f) for microgel elasticity \( E^* = 166 \text{Pa} \) (a) \( \phi = 0.56 \) & (d) \( \phi = 0.76 \), \( E^* = 179 \text{Pa} \) (b) \( \phi = 0.52 \) & (e) \( \phi = 0.68 \), and \( E^* = 2.6 \times 10^4 \text{Pa} \) (c) \( \phi = 0.49 \) & (f) \( \phi = 0.76 \).
To probe the length scales of heterogeneities associated with the transition from fragile to strong glass formers, correlations in the dynamics must be measured in two different points of time and space. Previous studies have achieved this through the use of the four-point correlation function, $g_4(ς, r)$, which calculates the variance of the fluctuations of the overlap order parameter. [94]

$$g_4(ς, r) = \frac{1}{N_ρ} \sum_{ijkl} \langle \delta(r - r_k(0) + r_i(0))w(|r_i(0) - r_j(ς)|)w(|r_k(0) - r_l(ς)|) \rangle - \langle w(|r_i(0) - r_j(ς)|) \rangle \langle w(|r_k(0) - r_l(ς)|) \rangle - \langle \frac{q_ς(ς)}{N} \rangle^2$$ (5.1)

Figure 5.5: The normalized four-point correlation function, $g_4^*(ς, r)$, at varied lag times, $ς$, for (a) $E^* = 166$Pa & $φ=0.61$ for $ς = 73$ (black), $ς = 730$ (red), $ς = 3.6x10^3$ (green), $ς = 7.2x10^3$ (blue), & $ς = 2.9x10^4$ s (cyan) and (b) $E^* = 2.6x10^3$Pa, $φ=0.52$ for $ς = 76$ (black), $ς = 760$ (red), $ς = 2.9x10^3$ (green), $ς = 1.2x10^4$ (blue), & $ς = 5.3x10^4$ s (cyan)
Here, a value of $a$ is chosen to be the distance to the primary peak of $g(r)$. This distance allows for the deformability of the microgels at high concentrations. The function is normalized by $q_s(\tau)$ in order to remove the time dependence of the function.

\[
g_4^*(r, \tau) = \frac{g_4(r, \tau)}{<q_s(\tau)/N>^2 - 1} \quad (5.2)
\]

Comparing $g_4^*(r, \tau)$ at varied lag times over the course of the observation period for $E^* = 166\text{Pa}$ and $E^* = 2.6 \times 10^4\text{Pa}$ in Figure 5.5(a-b) reveals that growth of dynamic heterogeneities will vary based on the elasticity of the particles. For soft particles, the dynamic correlations do not show any growth over the observation period. The stiff particles show an increase of dynamic correlations reaching a maximum at an intermediate time scale, and they will show a decrease at longer time scales.

A dynamic length scale, $\xi_4$, is extracted from $g_4^*(r, \tau)$ using an envelope fitting

**Figure 5.6:** The dynamic length scale, $\xi_4$, scaled by the effective radius, $r_{\text{eff}}$, for (a) $E^* = 166\text{Pa}$ $\phi = 0.47$ (black squares), $\phi = 0.60$ (red circles), $\phi = 0.75$ (green triangles), & $\phi = 0.81$ (inverted blue triangles) and (b) $E^* = 2.6 \times 10^4\text{Pa}$ $\phi = 0.49$ (black squares), $\phi = 0.52$ (red circles), $\phi = 0.56$ (green triangles), & $\phi = 0.58$ (inverted blue triangles)
method over a range of $2 \mu m < r < 9 \mu m$. [110]

$$g_4^*(r, \tau) = A \exp(-r/\xi_4)$$  \hspace{1cm} (5.3)

Here, $A$ is a free floating variable. Previous studies have successfully obtained relevant length scales using this fitting procedure. [94] This range of distance away from the center of the particle is chosen so multi-particle interactions will be probed. This length scale is a time-dependent property which reveals growth of the CRRs as they form in the super-cooled liquid. The maximum value observed is typically considered the equilibrium size of the CRRs. Scaling $\xi_4$ by the $r_{eff}$ normalizes the length scale for the difference in microgel size at each $\phi$. To observe the fragility effect on $\xi_4$, the lag time dependence of the scaled $\xi_4$ is shown in Figure 5.6 for microgel suspensions with elasticity’s $E^* = 166$Pa and $E^* = 2.6 \times 10^4$Pa. As expected, the larger length scales are observed in the fragile system by a magnitude of almost two particle radii. [109] Also evident from this figure is that CRRs grow gradually for the strong glass-forming systems and appear to grow more abruptly in fragile glass formers. Interestingly, for both microgel systems, there is a characteristic peak in $\xi_4$, defined as $\xi_4,\text{peak}$, at long lag times. This suggests the growth of CRRs to their maximum size is a slow process which may be fragility independent.

It is expected that the size of the CRRs increase as the glass-forming material approaches the phase transition between the super-cooled and glassy phases. Also, a maximum size of the CRR would be observed prior to the onset of the glassy phase. [109] Therefore, by fitting the $\phi$ dependence of $\xi_4,\text{peak}$, the maximum dynamic length scale, $\xi_4,g$, can be predicted for each microgel system. By normalizing $\xi_4,\text{peak}$ by $\xi_4,g$, the growth of CRRs with increasing $\phi$ is observed, shown in Figure 5.7 for each microgel
Figure 5.7: The dynamic length scale, $\xi_4$, normalized by $\xi_{4,g}$ at the glass transition, $\xi_{4,g}$, versus the volume fraction, $\phi$, scaled by the glass transition volume fraction, $\phi_g$, for $E^* = 166\text{Pa}$ (black squares), $E^* = 169\text{Pa}$ (red circles), $E^* = 179\text{Pa}$ (green triangles), $E^* = 436\text{Pa}$ (inverted blue triangles), and $E^* = 2.6 \times 10^4\text{Pa}$ (cyan diamonds)

It is immediately evident that the $\phi$ dependence of $\xi_4$ parallels the $\phi$ dependence of $\tau_0$ shown in Figure 4.8 in the previous section. This implies a direct correlation between dynamic heterogeneity and the differences between strong and fragile glass formers. The extended super-cooled regime observed in the phase diagram for strong glass formers is related to the strong directional motion in the deformable particles. For the fragile glass-forming systems, the sharp increase in $\xi_4$ is associated with the narrow range of the super-cooled phase.

Analysis of the four-point correlation function revealed that length scales associated with dynamical heterogeneity are fragility dependent. However, it did not provide a
picture of the shape of these clusters nor whether the nature of these clusters changes with glass fragility. To do so, first, the top 7% from the probability distribution of mobile particle is chosen as a definition for highly mobile particles. The locations of these mobile particles are shown in a particle map in Figure 5.8 for $E^* = 166\text{Pa}$ and $E^* = 2.6 \times 10^4\text{Pa}$ for concentrations in the super-cooled regime at lag times of $\tau = 7200\text{s}$. As with the Debye-Waller map, the distribution of highly mobile particles is spatially heterogeneous for both microgel systems. For both the strong and fragile glass-forming systems, there is visual evidence of string-like coordinated motion. There are many strings of 2-3 particles and some strings as large as 5-7 particles.

The map of mobile clusters revealed strong evidence to confirm that the CRRs move in a string-like fashion. Previous studies have shown that clusters of mobile particles contain replacing pairs of particles in quasi-1D motion. [93,111] This string-like motion is examined continuing to observe the top 7% most mobile particles at each lag time. These particles are tested by whether any two mobile particles, $i$ and $j$, satisfy the following criteria:

**Figure 5.8:** Particle map of the top 7% mobile particles for (a) $E^* = 166\text{Pa}$ & $\phi = 0.81$ and (b) $E^* = 2.6 \times 10^4\text{Pa}$ & $\phi = 0.55$. Colors correspond to change in position as indicated by the color pallet on the right.
Here, $\delta$ is kept as the distance to the primary peak of $g(r)$. This criterion examines, if one particle moves, whether or not an adjacent particle will replace it within a radial distance $\delta$. Therefore, it is essential that $\delta$ encompass the deformability of the particle so the particle size is not overestimated. This criteria is used to develop a probability distribution of string sizes, $P_s(n,\tau)$. The mean string size, $<L_S>$, is then quantified by calculating the weigh-average string size.

$$<L_S> = \frac{\sum_n n_S^2 P_s(n_S,\tau)}{\sum_n n_S P_s(n_S,\tau)}$$  \hspace{1cm} (5.5)

Here, $n_S$ is the string size in number of particles and $P_s(n_S,\tau)$ is the probability of observing the aforementioned string size. Similar to $\xi_4$, this variable is time-dependent and increases with lag time until $<L_S>$ reaches a maturation point. The lag time

Figure 5.9: The average string size, $<L_S>$, versus lag time, $\tau$, for (a) $E^* = 166$Pa: $\phi = 0.32$ (black squares), $\phi = 0.56$ (red circles), $\phi = 0.60$ (green triangles), $\phi = 0.75$ (inverted blue triangles), & $\phi = 0.81$ (cyan diamonds) and (b) $E^* = 2.6 \times 10^4$Pa: $\phi = 0.49$ (black squares), $\phi = 0.50$ (red circles), $\phi = 0.55$ (green triangles), & $\phi = 0.58$ (inverted blue triangles).
dependence of $<L_s>$ is shown in Figure 5.9 for microgel elasticity $E^* = 166\text{Pa}$ and $E^* = 2.6\times10^4\text{Pa}$. Contrary to the behavior of $\xi_4$, $<L_s>$ displays a characteristic peak, denoted as $<L_s>_{\text{peak}}$, at much shorter time scales suggesting the strings reach a maximum size much faster than the dynamic length scale. This lag time behavior has interesting implications as it may suggest that strings form and dissociate inside of these mobile clusters as they grow in size. It is also evident from this figure that the string sizes are larger in the strong glass-forming systems than in the fragile glass-forming system. This evidence further supports that there is strong directional motion in strong glass-forming liquids.

Looking at the $\phi$ dependence $<L_s>_{\text{peak}}$, shown in Figure 5.10, there is further evidence that the string size is dependent on the glass fragility. The largest strings are observed for

![Figure 5.10: The peak string size, $<L_s>_{\text{peak}}$, versus volume fraction, $\phi$, scaled by the glass transition volume fraction, $\phi_g$, for $E^* = 166\text{Pa}$ (black squares), $E^* = 179\text{Pa}$ (green triangles), $E^* = 436\text{Pa}$ (inverted blue triangles), and $E^* = 2.6\times10^4\text{Pa}$. The lines are the corresponding linear fits to the string growth.](image)
the strongest glass formers and decreases with increasing elasticity. For each microgel system, the rate of growth of strings with increasing $\phi$ is relatively similar. However, the onset of string growth is strongly dependent on the microgel elasticity. Similar to the $\phi$ dependence of $\xi$$_\text{s}$, this behavior results from the extended super-cooled regime as observed in strong glass-forming liquids. This likely leads to the growth of longer strings that are observed in the strong glass-forming systems. Through a linear fit of the string growth, the maximum string size, $<L_s>_{\text{max}}$, is predicted for each microgel system. These values are plotted against the fragility index in Figure 5.11. This shows a direct parallel with the glass fragility and string size.

While the previous analysis determined the average string sizes in the glass-forming material, it is also of interest to measure the probability distribution of string sizes, $P(L)$, to determine if the maximum string length is dependent on the glass fragility. The

Figure 5.11: The maximum string size $<L_s>_{\text{max}}$ (dark green pentagons) and the fragility index (dark red hexagons) versus microgel elasticity, $E^*$. 
distributions for microgel systems with elasticity $E^* = 166\text{Pa}$ and $E^* = 2.6 \times 10^4\text{Pa}$ are shown in Figure 5.12. These figures show that for the strong glass-forming suspensions, there are a small percentage of strings that are much longer than the assembled average. This coincides with the map of highly mobile particles shown in Figure 5.8. For strong glass-forming suspensions, there is evidence of string lengths of 6-8 particles for ~1% of the population of highly mobile particles. For the fragile suspension, this length is nearly half with a string size of 4-5 particles for the same percentage of the population. This coincides with the analysis of $<L_s>$ that strings are longer in strong glass-forming systems due to their strong directional motion.

For both the strong and fragile glass-forming system, there is an exponential decay in $P(L)$ with increasing string length. A recent study showed that this string-like motion

![Graph](image)

**Figure 5.12:** The probability distribution of strings $P(L)$ versus string length, $L$, for (a) $E^* = 166\text{Pa}$: $\phi = 0.32$ (black squares), $\phi = 0.56$ (red circles), $\phi = 0.60$ (green triangles), $\phi = 0.75$ (inverted blue triangles), & $\phi = 0.81$ (cyan diamonds) and (b) $E^* = 2.6 \times 10^4\text{Pa}$: $\phi = 0.49$ (black squares), $\phi = 0.50$ (red circles), $\phi = 0.55$ (green triangles), & $\phi = 0.58$ (inverted blue triangles).
behavior parallels the behavior of “living polymers” in solution. [112] Each particle in the string acts as the monomer unit of the polymer. For strings with a length of ~4 or less, the strings are considered to be self-avoiding walk (SAW) polymers. [112] Above that critical length, the strings take the form of a random walk (RW) polymer. [112] Looking back at $<L_S>$ peak for each microgel system, it is evident that the average string size observed in this analysis behaves as a SAW polymer. However, looking at $<L_S>$ peak for the strong glass-forming system, it appears that the average string size would transition from the behavior of SAW polymer to the behavior of an RW polymer. Using this definition, it is possible to develop a better understanding of the nature of the string-like motion.

5.5 Conclusion

Mobile and immobile clusters are heterogeneously distributed throughout glass-forming liquids in the super-cooled phase. These clusters grow in size as the material approaches the glass transition. As expected from previous studies, the dynamic length scales that distinguish these CRRs are larger in fragile glass-forming liquids. The growth of these length scales is also fragility dependent, which a gradual growth observed in strong glass-formers and an abrupt growth is observed in fragile glass-formers. The CRRs are characterized by string-like particle motion and the string-sizes are slightly larger in strong glass-formers. This is most likely due to strong directional motion of particles in strong-glass forming liquids. Based on the probability distribution of string sizes, strings can be described as living polymers, which provides a better picture of their shape and dynamics in the microgel suspension.
6.1 Introduction

In this chapter, the dynamics of highly deformable microgel particles is investigated under spatial confinement through a direct confocal microscopic study to visualize inter-particle dynamics. The goal of this work is to determine confinement length scales for strong glass-forming liquids and measure how they deviate from length scales for hard-sphere suspensions. It is also investigated whether dynamic length scales change with decreasing confinement gap size in order to develop a better understanding of the relationship between CRRs and the confinement effect.

6.2 Background

Introduced in Chapter 1, spatial confinement of glass forming liquids can modify the onset of the glass transition. When there are strong interactions with the confining surface, the confinement effect will induce the glass transition. Furthermore, it is expected that the confinement effect occurs when the confining length scale is less than
the characteristic size of the CRRs. [42] One interesting aspect of confinement of glass-forming suspensions is the strengthening of glass fragility. This was observed for strong glass-forming liquids, e.g. polymer films and super-cooled water. [43,113] It is conjectured that the deviations in glass fragility result from weak packing frustrations under small spatial confinement. Fragile glass-forming liquids in the bulk will transition to strong glass-forming behavior. [43] Consequently, confinement is expected to have a weak effect on the fragility of strong glass-forming liquids. For strong glass-forming polymer films, bulk behavior is observed down to confinements of 30-40nm which translates to ~20 particle diameters. [115-116]

With regard to dynamic heterogeneity, the size of CRRs grows significantly with decreasing confinement gaps for fragile systems. [93-94] Both the dynamic length scale and the string length exhibit a 2-3 fold increase as small confinement gaps. In contrast, these finite-size effects due to confinement are only minimally observed in strong glass-forming liquids. [43] There is only a minor change in the CRRs for strong glass-formers even at small confinement gaps, with only minor deviations in CRRs near the confining surface. Due to the opposing behavior of strong and fragile glass-forming liquids, it is suggested that the finite-size effects are strongly dependent on the glass fragility. [43] The deviations in CRRs in strong glass-forming liquids at the confining surface are likely dependent on surface interactions. [117] A strongly attractive confining surface will percolate immobile clusters into the bulk, which will decrease the probability of observing a mobile cluster.

Shown in Chapters 4 and 5, colloidal glasses are model systems for studying glassy dynamics due to their observable size via microscopic methods, e.g. wide-field
microscopy and confocal microscopy. [41,118-119] Recent studies have also shown that the particle dynamics in confined colloidal glass-forming liquids parallels the behavior for confined molecular liquids. [93-94] Hard-sphere suspensions, at low particle concentrations, such as $\phi=0.40$ and $\phi=0.43$, exhibit slow dynamics and becomes comparable to the glass transition when the boundary layers are reduced to 10-30 particle diameters. The exact value of the critical thickness at which structural arrest is achieved is dependent on the particle concentration. [93] Furthermore, there is evidence to suggest that confinement leads to an increase in dynamic heterogeneities due the presence of CRRs. Dynamic length scales for a range of concentrations, $\varphi=0.40-0.57$, increased as the confinement gap is reduced, and were smaller than the critical thickness at all concentrations. [94] It was further illustrated that these CRRs are characterized by string-like particle motion. [93] As the film thickness is reduced, the string size becomes more pronounced, implying that the fragility of the suspension becomes stronger with confinement.

In this chapter, fluorescent microgel particles of low elasticity are confined to small length scales in order to expound upon the confinement effect of strong glass-forming liquids. At each incremental gap, the particle mobility and distribution is measured via image processing and particle-tracking algorithms described previous chapters. As expected, it is reported here that particles in strong glass-forming suspension remain mobile at much smaller confinement gaps than what is reported for hard-sphere suspension. Dynamic length scales remain nearly constant at decreasing confinement gaps, confirming the fragility dependence of the confinement effect on CRRs.
6.3 Experimental

6.3.1 Materials

Fluorescent PNIPAM microgel particles used for this work are synthesized using the procedure detailed in Chapter 2. [49] Based on the analysis in Chapter 3, microgels with a CL ratio of 1.6% are chosen due to their strong glass-forming behavior with a fragility index of $m = 4.5$. These microgels have an elasticity constant of $E^* = 169$Pa as determined by the analysis of the Hertzian model. Targeted concentrations are achieved by concentrating the microgel suspensions using a Rotovap R-210 at 60°C and redilutions with deionized water. Time series images are acquired using confocal microscopy (Zeiss LSM 5 Pascal, 100x objective, NA=1.4).

6.3.2 Confinement of Colloidal Suspensions

A custom-built compression apparatus, shown in Figure 6.1, is mounted directly onto the sample stage of the confocal microscope. The sample is loaded directly to a cover slip and a quartz cylinder is lowered from the top by turning the adjustments on the device. Both confining surfaces are roughened in order to prevent crystallization of the sample. A very polydisperse sample of PNIPAM microgels is spin-coated onto the glass coverslip and quartz cylinder and sintered at 80°C. This will prevent localized crystallization of the suspension at small confinements. Once the sample is loaded into the apparatus, the desired film thickness is acquired by systematically using the fine and two coarse micrometers. Decreasing the film thickness is achieved in a step-wise fashion by lowering the top at a compression rate of ~10μm/min, waiting ~5min between each
compression step to minimalize drift. Typically, the first measurement is performed at a large gap size, to capture bulk behavior, and the confinement height is lowered to incremental smaller gap sizes before each following measurement. Film thickness is determined by the confocal z-stack profiles with an accuracy of ±0.1 μm. After achieving the desired film thickness, the sample is left undisturbed for ~30min before commencing measurements. All measurements are performed at room temperature, T = 25°C, where the microgels are swollen in solution.

Measurement acquisition and analysis is accomplished using the IDL particle algorithms detailed in Chapter 3. The 3D image size is typically 30μm x 30μm in the x-y directions; however, the size in the z-direction is dependent on the gap size. For bulk and large gaps, the size of the image in the z-direction is 12μm. For smaller gaps, this gap size is reduced to ensure that the particles under analysis are at least 2 particle diameters
from the confining walls. As with the previous analysis, it is expected that the tracking algorithms will determine the center of the particle with an accuracy of 0.05 μm in the x-y plane and 0.2 μm in the z-direction.

6.4 Results & Discussion

To examine the liquid and super-cooled phases for a strong glass-forming liquid, three volume fractions of microgels, \( \phi = 0.49 \), \( \phi = 0.56 \), and \( \phi = 0.70 \), with an elasticity of \( E^* = 169 \text{Pa} \), are prepared for this study. These volume fractions are calculated for the bulk suspensions using the model introduced in Chapter 4 with Equations 4.2 and 4.3. Based on the phase diagram shown in Figure 4.7, it is expected that the suspension with \( \phi = 0.49 \) will exhibit liquid-like behavior and the suspensions with \( \phi = 0.56 \) and \( \phi = 0.70 \) will exhibit super-cooled phase behavior. The confinement height is reported as \( h/d_H \) where \( h \) is the confinement height and \( d_H \) is the hydrodynamic particle diameters. This is chosen so the values would be uniform between concentrations. Therefore, confinement is measured in unperturbed particle diameters.

First, due to the deformability of soft microgels, the effect of confinement pressure on the size of the microgel is analyzed through the pair correlation function, \( g(r) \), introduced in Chapter 4 as Equation 4.1. Figure 6.2 shows \( g(r) \) for three volumes fractions for various confinement heights. In the liquid phase, \( \phi = 0.49 \), the first peak of \( g(r) \), shown in Figure 6.2(a), shown no deviations even down to a gap size of \( h/d_H = 11 \). For \( \phi = 0.56 \), shown in Figure 6.2(b), the first peak remains stable but does show a slight shift to smaller separation distances. The secondary and tertiary peaks for \( h/d_H = 18 \) show a more significant shift. After increasing the volume fraction further to \( \phi = 0.70 \), shown in Figure
Figure 6.2: The pair correlation function, g(r), for (a) $\phi = 0.49$: bulk (black), $h/d_H = 16$ (red), & $h/d_H = 11$ (green); (b) $\phi = 0.56$: bulk (black), $h/d_H = 30$ (red), $h/d_H = 22$ (green), & $h/d_H = 18$ (blue); (c) $\phi = 0.70$: bulk (black), $h/d_H = 52$ (red), $h/d_H = 22$ (green), $h/d_H = 16$ (blue), & $h/d_H = 14$ (cyan)
6.2c, the shift of the peaks of $g(r)$ from bulk behavior are much more significant for gap heights of $h/d_H = 16$ and $h/d_H = 14$. These results suggest that the confinement effect influences the effective size of the microgels. The onset of this effect appears to occur at a gap height of $\sim 20h/d_H$, which is smaller than reported values for the confinement effect in hard-sphere suspensions.

Next, it assessed how confinement influences the mobility of the soft microgel suspensions. As with the bulk analysis in Chapter 4, the particle mobility is quantified by measuring the mean-square displacement (MSD), $<x^2>$, defined as Equation 4.5, which is an assembled average of all particle motion over the x-coordinate. [113] Figure 6.3 shows the computed $<x^2>$ versus lag time, $\tau$. The liquid phase, shown in Figure 6.3a for $\phi = 0.46$, shows no change in particle mobility as the gap is reduced from the bulk to $h/d_H = \ldots$

**Figure 6.3:** The mean-squared displacement, $<x^2>$, as a function of lag time, $\tau$, for (a) $\phi = 0.49$: bulk (black squares), $h/d_H = 16$ (red circles), & $h/d_H = 11$ (green triangles) and (b) $\phi = 0.70$: bulk (black squares), $h/d_H = 30$ (red circles), $h/d_H = 22$ (green triangles), $h/d_H = 16$ (blue triangles), & $h/d_H = 14$ (cyan diamonds).
11. This suggests that the confinement effect has very little influence on the particle motion in the liquid phase. In the super-cooled phase, shown in Figure 6.3(b) for $\phi = 0.70$, there is an observed deviation from bulk behavior with decreasing gap height from the bulk down to $h/d_H = 14$. However, this deviation is considerably moderate when compared to the sharp decrease in mobility observed for hard-sphere systems. [43,114-115] Returning to the pair correlation results, it is inherent that there is a link between the decreasing size and prolonged particle mobility at small confinement gaps. The glass transition in colloidal suspensions is a jamming process. If soft microgel particles deform at small confinement gaps, this would prevent the suspension from becoming jammed.

With evidence of unimpeded particle mobility at small confinement gaps, it is now of interest to determine how confinement influences the structural relaxation in super-cooled strong glass-forming liquids. This is accomplished by calculating the overlap-order parameter, $q_s(\tau)$, defined by Equations 4.7(a-b) in Chapter 4, for the suspension with a concentration of $\phi = 0.70$, shown in Figure 6.4(a). As with the previous analysis, the separation distance, $a$, is defined as the $0.5d_H$ to distinguish between localized and delocalized particles. Similar to results of the MSD calculations, there is a moderate slowing down in particle relaxation induced by decreasing the confinement gap. The deviation from bulk behavior is much less severe than what was observed in hard-sphere suspensions [43,114-115] Looking at the decay of $q_s(\tau)$, there is evidence to suggest a gradual increase in relaxation times to $10,000s$, an order of magnitude less than characteristic relaxation time, $\tau_g$, of the glass transition. For the smallest gap size, $h/d_H = 14$, the decay should occur at a timescale much longer than $10,000s$ but less than $\tau_g$. 

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Figure 6.4: (a) The overlap-order parameter, $q_S(\tau)$, versus lag time, $\tau$, for $\phi=0.7$: bulk (black squares), $h/d_H = 30$ (red circles), $h/d_H = 22$ (green triangles), $h/d_H = 16$ (blue triangles), & $h/d_H = 14$ (cyan diamonds) (b) the scaled alpha-relaxation time, $k_{\tau_{\alpha}}$, versus confinement height, $H$, scaled by the hydrodynamic radius, $d_H$. 
Relaxation times are extracted from $q_s(\tau)$ using the Kohlrausch-Williams Watts (KWW) equation, defined by Equation 4.8 in Chapter 4. The $\alpha$-relaxation time, $\tau_{\alpha}$, versus the confinement gap is shown in Figure 6.4(b) for $\phi = 0.70$. These results confirm the behavior observed in $q_s(\tau)$. There is a gradual increase in $\tau_{\alpha}$ from $\sim 5000s$ to $\sim 10,000s$ as the confinement gap is decreased from $h/d_H \sim 100$ to $h/d_H \sim 16$. Below that gap size, there is a sharp increase in $\tau_{\alpha}$ to a value of about $\sim 40,000s$. The spike in $\tau_{\alpha}$ can be attributed to the confinement length scale. Analysis of hard-sphere suspensions revealed a confinement length scale around $\sim 30$ to $\sim 40$ particle diameters, which is significantly greater than the confinement length scales observed for these soft microgel suspensions. However, when the shrinkage of the deformable microgels is taken into account, the effective size, $r_{\text{eff}}$, of the microgel at $h/d_H = 14$ is $\sim 93\%$ of $r_{\text{eff}}$ in the bulk and $\sim 82\%$ of the hydrodynamic radius, $R_H$. Therefore, the confinement gap can be recalculated to be less than $h/r_{\text{eff}} \sim 19$, which is still significantly less than the confinement length scale for the hard-sphere suspension.

With evidence that confinement of suspensions of deformable microgel particles gradually induces the glass transition, it is now the aim of this study to determine if this confinement effect influences dynamic heterogeneity in strong glass-forming suspensions. First, it is explored how confinement modifies the four-point correlation function, $g_4^*(\tau,r)$, as defined by Equations 5.1 & 5.2 in the previous chapter. Figure 6.5(a-d) shows $g_4^*(\tau,r)$ for incremental lag times for decreasing confinement gaps. As expected, there is a marginal decrease in the location of the peaks with decreasing confinement due to particle deformation. More remarkably, even at confinements below
Figure 6.5: The normalized four-point correlation function, $g_4^*(\tau,r)$, at varied lag times, $\tau$, for $E^* = 169\text{Pa} \& \phi = 0.7$ for confinement heights: (a) $h/d_H = 52$, (b) $h/d_H = 22$, (c) $h/d_H = 16$, & (d) $h/d_H = 14$. Colors correspond to lag times, $\tau = 75.1\text{s}$ (black), $\tau = 751\text{s}$ (red), $\tau = 1.88\times10^3\text{s}$ (green), $\tau = 7.59\times10^3\text{s}$ (blue), & $\tau = 1.32\times10^4\text{s}$ (cyan).
the apparent onset of the confinement effect, there is no significant change in the behavior of $g_4(\tau,r)$.

Using the envelope fitting method detail in the previous chapter with Equation 5.3, the dynamic length scale, $\xi_4$, is extracted for each confinement gap. For the confinement gap dependence of $\xi_4$, shown in Figure 6.6(a), there is a scatter of $\xi_4$ with decreasing confinement gap heights. When these values are normalized by the center-to-center separation distance, determined from the first peak of $g(r)$, shown in Figure 6.6(b), $\xi_4$ appears to be unaffected by the confinement gap with an average value of 2.24 particle diameters with a standard deviation 0.43, which is within the range of acceptable error. This is further evidence that the deformability of microgels is a main contributor to how these suspensions maintain bulk behavior at small confinement gap sizes. Interestingly, as microgels shrink in size and become more dense, their elasticity increases. This would eventually lead to hard-sphere particles, which would exhibit fragile glass-forming behavior in bulk suspensions. However, as fragile glass-forming liquids strengthen under confinement, these strong glass formers are expected to maintain their fragility. [43]

6.5 Conclusions

It is demonstrated here that confinement has a minimal effect on dynamics of suspensions of highly deformable microgels. Suspensions in the liquid phase and early in the super-cooled phase show nearly constant mobility even down to very small gaps of ~10 particle diameters. Decreasing the confinement gaps leads to shrinking of the microgels preventing the onset of a jammed state, which maintains the bulk mobility. At
Figure 6.6: (a) The dynamic length scale, $\xi_4$, as a function of confinement height, $H/d_H$. (b) Same as (a) with $\xi_4$ scaled by the separation distance, $R_0$. 
higher concentrations in the super-cooled phase, there is a marginal decrease in particle mobility. The dependence of $\tau_a$ on gap size reveals the onset of confinement effect may occur at a height range less than of 14-19 particle diameters. This value is much less than the reported values for hard-sphere suspensions. Finally, there is no observable change in dynamic heterogeneity due to the confinement effect for the strong glass-forming suspensions.

6.6 Future Work

The results here provide insight into the confinement effect on strong glass-forming suspensions. This accompanies the previous work on fragile glass forming suspensions to detail the confinement effect on opposite ends of fragility spectrum. However, there is a gap in information regarding confinement effect on intermittent glass fragilities. It is of interest to continue this work for microgel suspensions of variable elasticity to elucidate the transition the fragility dependence of the confinement effect. As demonstrated in Chapter 4, glass fragility is controlled by the microgel elasticity. Examining the confinement effect on the range of microgel elasticity would reveal the transition of confinement effect for strong to fragile glass forming liquids. It would be interesting to see if this transition occurs continuously or if a critical fragility exists at which transition occurs abruptly. This study would need to observe both the relaxation dynamics specified in Chapter 4 and the dynamic heterogeneity discussed in Chapter 5. Measuring how relaxation times and dynamic length scales vary for each confined glass-forming suspension would reveal the underlying phenomena involved with confinement effect and help explain why it varies between strong and fragile glass-forming liquids.
CHAPTER 7:
CONCLUSIONS & PERSPECTIVES

Microgels are continually proven to be powerful materials for an expanse of applications for industry. With broad functionality, microgels are also able to elucidate aspects of fundamental sciences. In the previous chapters, microgels were employed for both of these purposes. Chapter 2 demonstrated how microgel synthesis is tunable to capture the properties of globule proteins as well as act model colloidal particles with variable elasticity. Two microgel systems, based on PNIPAM and PMSA, were successfully synthesized using emulsion polymerization techniques. Both polymer backbones are stimuli response with LCST behavior at 32°C for PNIPAM and UCST behavior at 65°C for PMSA. There is an abundance of future work available in the field of microgel synthesis. First, it is of interest to explore additional monomer backbones. Monomers with different functional groups or charge groups would provide new properties for both areas of research discussed in this dissertation, including enhanced biocompatibility or improved viscoelasticity. Furthermore, as mentioned in Chapter 2, there are four synthesis techniques commonly employed to synthesize microgels. It
would also be of interest to explore these additional synthesis methods to create microgels with novel properties.

Demonstrated in Chapter 3, microgels are a biomimicry of the globular proteins, and they were successfully employed to design a super-lubricant inspired by the synovial fluids. Mixtures of PNIPAM and PMSA microgels with the biopolymer Hyaluronic Acid exhibited the viscoelastic properties and low friction behavior of the Synovial Fluids. While this study provides significant evidence that these lubricants match the material properties of the Synovial Fluids, there are many directions to take this project in the future. For example, the friction behavior of these lubricants could be further explored. The nano-tribological study provided strong evidence of the low frictional behavior of these mixtures. A large-scaled tribological measurement to further test the mechanical wear of these mixtures would provide further support to lubrication properties of these microgel-biopolymer mixtures. Secondly, the lubricants could be further explored as a remedy for arthritis and joint wear. This would require an in-depth biomedical study of the interactions of the biological components in the Synovial joints with microgel particles. Biocompatibility and biotribology of these mixtures need to be studied extensively to confirm these mixtures as an effective remedy.

Chapters 4-6 showed how suspensions of microgel particles can be employed to elucidate the physical phenomena associated with the glass transition. The particle tracking analysis of the microgels of variable elasticity revealed a transition from fragile to strong glass-forming behavior as microgels deviate from hard-sphere behavior and become more deformable. The presence of dynamic heterogeneity in the form of cooperatively rearranging regions is predominant in all microgel suspensions analyzed.
The growth of these regions is directly linked to the glass fragility, with a more gradual growth in strong glass-forming liquids. Confining a strong glass-forming microgel suspension revealed only a minor deviation from bulk behavior at small confinements. Future work in this project is primarily focused in the area of confinement. As specified in the Chapter 6, it is of interest to investigate the confinement effect on the full spectrum of microgel elasticity analyzed in Chapters 4 and 5. This will help develop a better understanding of confinement and dynamic length scales and how they relate to glass fragility. There is also interest to measure viscoelastic properties of these microgel suspensions. This study would relate the microscopic and macroscopic properties of the glassy materials and how glass fragility is expressed in the bulk material. This is important to understand for industries involving glassy materials as many material properties, e.g. viscosity, will govern production parameters and rates.

Overall, this dissertation presents a small portion of the vast potential of microgel particles. Not only are they highly applicable, they make great probes to help solve many unanswered questions in science. There are many directions to explore with this work in the future, which could lead to some exciting research and novel inventions. The groundwork laid in this dissertation could help initiate a variety of future studies and developments involving microgel particles.
REFERENCES


[99] Our analysis shows that $\langle x^2 \rangle \approx \langle y^2 \rangle$, which is not shown here. Because of slower scanning along the z-direction, we focus on the in-plane particle dynamics parallel to the confining walls (the xy plane) in this work.


