

Formation of Magnetite Clusters using a Confined Impinging Jet Mixer

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Superparamagnetic nanoparticles (SPM NP) are commonly employed as magnetic resonance imaging contrast agents. Magnetite (Fe_3O_4) is of increasing interest because of its high magnetic susceptibility in an applied magnetic field. It has been shown in the literature that an increase in size of transverse NMR relaxivity agents leads to a significant decrease in T2 relaxation times and thus higher contrast in an MRI. It is therefore important to develop methods and conditions for controlling the size of polymer-stabilized magnetite clusters. Our strategy is to elucidate critical coagulation concentrations of water for magnetite particles that have been coated with an amphiphilic poly(propylene oxide-*b*-ethylene oxide)-OH (PPO-*b*-PEO) copolymer to control clustering size through the use of a confined impinging jet (CIJ) mixer. Using the critical coagulation concentrations, operating conditions for the CIJ mixer have been found where micellization of the copolymer occurs simultaneously with magnetite clustering. Preliminary results have shown that control over the size distribution may be possible by changing the concentration of water in the mixing chamber.

Introduction

The study of superparamagnetic nanoparticles (SPM NP) has led to the potential employment of polymer-coated nanoclusters in the areas of drug delivery ⁽¹⁾, magnetic resonance imaging (MRI) relaxivity agents ⁽²⁾, bio-catalysis, and bio-separations ⁽³⁾. Gadolinium, cobalt, and magnetite are common SPM NPs used in biological systems. Magnetite (Fe_3O_4), being an iron oxide, is well suited for oxygen-rich environments and will not lose its superparamagnetic properties over time due to oxidation ^(4; 5). For particle sizes less than 25 nm, the thermal energy is stronger than the magnetic coupling forces so there is no longer a net magnetic moment ^(6; 7; 8; 9; 10). When a magnetic field is applied, the magnetic coupling forces once again dominate and all particles align with the magnetic field ⁽¹¹⁾.

MRI contrast agents represent a particularly useful application of polymer-stabilized magnetite clusters. Magnetite is interesting for MRI as it has a larger magnetic moment in an applied image field ⁽¹²⁾. In MR imaging, a magnetic field is applied in the presence of time-varying radiofrequency fields. At equilibrium, the hydrogen atoms in the body are aligned with a net magnetization in the direction of the applied magnetic field. When the radiofrequency fields are applied, the alignment of

the atoms is disturbed. The time for the atoms in the body to return to equilibrium is called the relaxation time. There are two types of relaxation of hydrogen atoms measured in MRI, T1 and T2. T1 is the spin-lattice or longitudinal relaxation time and T2 is the spin-spin or transverse relaxation time. T1 and T2 occur and are measured simultaneously. Relaxivity is the inverse of relaxation time, so a smaller T1 or T2, leads to a higher relaxation, R1 or R2, respectively.

SPM NP, including magnetite, can be used as T2 contrast agents ⁽¹³⁾. It has been found that an increase in the size of MRI T2 contrast agents leads to a significant decrease in the T2 relaxation time ⁽⁵⁾. Magnetite particles greater than 25 nm in diameter do not exhibit superparamagnetic behavior, a prerequisite for functioning as a contrast agent. However, by forming clusters of smaller particles, superparamagnetism may still be maintained while also allowing for a larger size of the contrast agent.

In the presence of a magnetic field the magnetic moments of the particles align with the field. As water molecules diffuse randomly around the SPM NP they experience a range of field conditions ⁽¹⁵⁾. Polymer stabilization of the SPM NP is crucial for proper design of contrast agents. However, before polymer-stabilized magnetite nanoclusters can be employed for use in biological systems the conditions for optimal cluster formation must be elucidated.

To form polymer-stabilized magnetite nanoclusters we investigated the use of oleic acid versus poly(butylene oxide) (PBO) stabilized magnetite nanoparticles to determine which coating would allow for the better control of cluster size. Once the best coating for the magnetite was found, the critical water concentrations for magnetite nanoparticles and a diblock copolymer needed for cluster formation were examined and an operating region for confined impinging jet mixing was found for forming stabilized magnetite clusters.

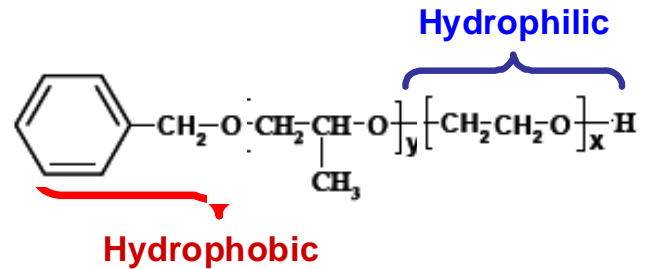


Figure 1: PPO-b-PEO

(x=34, y= 9)

MW = 2,000 g/mole

Methods and Materials

Synthesis of Coated Magnetite Nanoparticles

Oleic-acid coated magnetite nanoparticles with a narrow particle size distribution were synthesized in the Riffle group by the method outlined by Pinna et al. and adapted by Nikorn Pothayee ⁽¹⁶⁾. Iron(III) acetylacetonate is added to deoxygenated benzyl alcohol and slowly heated over the course of two hours to 205°C and then kept stable for 24 hours. The resultant black particles are washed with acetone, dried, and then redispersed in chloroform with oleic acid. The chloroform is evaporated and the particles are washed to remove any remaining oleic acid. Particle sizes are measured to be on the average diameter of 5.1 nm with dynamic light scattering (DLS) and average radius of 3.8 nm with transmission electron microscopy (TEM) ⁽¹⁷⁾.

Poly(butylene oxide), PBO, coated magnetite was synthesized in the Riffle group. PBO was made by reacting 1,2-epoxybutane initiated with 3-hydroxypropyl trivinylsilyl in a pressure reactor. The vinyls on the PBO fluid were then reacted with mercapto acetic acid in chloroform to functionalize the PBO with carboxylic acids. The magnetite was formed via coprecipitation of Fe₂Cl₃ and FeCl₃ with 50% ammonium hydroxide while mixed with an ultra-high speed homogenizer. The functionalized polymer was then dispersed in chloroform and added to the magnetite. The PBO adsorbs onto the magnetite particles via the carboxylic acid

groups. The complex is then placed in a separation funnel and allowed to separate, washing with chloroform. The chloroform is then evaporated off with a rotational evaporator to leave a PBO ferrofluid.

Amphiphilic Diblock Copolymer

The amphiphilic diblock copolymer stabilizer is a benzyl alcohol initiated poly(propylene oxide-*b*-ethylene oxide)-OH (Bz-PPO-*b*-PEO-OH) as shown in Figure 1. PPO has been used safely in cell studies and PEO has been approved by the FDA ⁽¹⁸⁾. The PPO is hydrophobic so it interacts with the hydrophobic magnetite coating. The PEO is hydrophilic, providing stabilization in the aqueous antisolvent.

Derjaguin, Landau, Verwey, Overbeck (DLVO) Theory

Derjaguin, Landau, Verwey, Overbeck (DLVO) theory proposes that the stability of colloidal systems can be described by the following:

$$V_T = V_{vdW} + V_{ES} + V_S + V_M \quad \text{Equation (1)}$$

V_T is the total potential, V_{vdW} is the van der Waals attraction, V_{ES} and V_S are electrostatic and steric repulsions, and V_M is the magnetic attraction ^(19; 20). DLVO theory can be used to predict the particle size needed for the magnetic forces to have a large enough range that steric repulsion is overcome in the presence of a magnetic field ^(19; 20). Using DLVO theory, conditions such as desired diblock molecular weight and necessary polymer loading for a specific cluster size can be estimated.

Critical Coagulation Concentration

To find the amount of water needed for cluster/micelle formation 1mL coated-magnetite solutions (magnetite-oleic acid or PBO-magnetite) or diblock copolymer was placed in a cuvette. Microliter amounts of water were added and dynamic light scattering (DLS) was used to determine when clustering or micellization occurred.

Synthesis of Magnetite Clusters

Magnetite clusters contained in polymer micelles are formed by the use of the Flash NanoPrecipitation technique (21: 22). The Flash NanoPrecipitation technique requires rapid mixing of the magnetite and an amphiphilic polymer suspended in an organic solvent with an appropriate antisolvent, which in this case is deionized (DI) water.

The Flash NanoPrecipitation method is carried out in a confined impinging jet (CIJ) mixer. For the CIJ process the contents of two syringes – one holding the magnetite and copolymer stabilizer suspended in the organic solvent, and the other holding the aqueous solvent – are injected into a mixing chamber as shown in Figure 2.

CIJ mixing is more effective at forming polymer-stabilized clusters than traditional mixing because it allows for very rapid mixing at a controlled and maintained weight percent of antisolvent in the mixing chamber (17).

The CIJ mixer works on the basis of mixing on three different scales: macromixing, mesomixing, and micromixing (21).

Macromixing occurs on the scale of the mixing vessel which, in the case of the present CIJ mixer is 0.109 inches, the mixing chamber diameter. Mesomixing occurs on the scale of turbulent eddies caused by the mixing of the two fluids. Micromixing

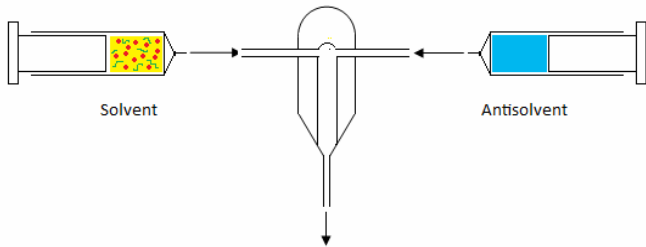


Figure 2: Confined Impinging Jet Mixer

occurs on the molecular level by diffusion of molecules across laminar regions. The equation for the momentum diffusivity (kinematic viscosity) of the liquid in the mixing chamber, i.e. the effluent from the mixer, v_3 , is shown below:

$$v_3 = \frac{(0.5\lambda_K)^2}{\tau_{diffusion}} \quad \text{Equation (2)}$$

where $\tau_{diffusion}$ is the time for diffusion of molecules from one fluid to the other. λ_K is the Kolmogorov length, the smallest length scale for turbulent flow, described by (5):

$$\lambda_K = \left[\frac{v_3}{\varepsilon} \right]^{1/4} \quad \text{Equation (3)}$$

ε is the energy dissipation rate, described as the rate of energy that is put into a system, P , divided by the mass over which the energy is dissipated. The mass is the the product of the density of the effluent stream and its volume so that:

$$\varepsilon = \frac{P}{\rho_3 V_m} \quad \text{Equation (4)}$$

$$P = \left(\frac{1}{2} m_1 u_1 + \frac{1}{2} m_2 u_2 \right) \quad \text{Equation (5)}$$

The mixing time is proportional to the time for diffusion (21).

$$\tau_m \propto \tau_{diffusion} = \frac{(0.5\lambda_K)^2}{v_3} \quad \text{Equation (6)}$$

Combining the above Equations (2) through (5) and substituting into Equation (6) gives a new relationship for the mixing time.

$$\tau_m \propto \frac{1}{4} \left(\frac{v_3}{\varepsilon} \right)^{1/2} = \frac{v_3^{1/2} \rho_3^{1/2} V_m^{1/2}}{4 \left(\frac{1}{2} m_1 u_1 + \frac{1}{2} m_2 u_2 \right)^{1/2}}$$

Equation (7)

In order for the streams to meet in the center of the chamber, the momenta of the streams must be matched.

$$\dot{m}_1 u_1 = \dot{m}_2 u_2 \quad \text{Equation (8)}$$

The mass flowrate (kg/s) of the fluid is \dot{m} and the linear velocity is u (m/s).

The mass flowrate of the fluid can be found by multiplying the volumetric flowrate, Q , by the density of the fluid, ρ .

$$\dot{m}_1 = Q_1 \rho_1 \quad \text{Equation (9a)}$$

$$\dot{m}_2 = Q_2 \rho_2 \quad \text{Equation (9b)}$$

The volumetric flowrate is the linear velocity multiplied by the cross sectional area, A , of the needle.

$$Q_1 = u_1 A_1 \quad \text{Equation (10a)}$$

$$Q_2 = u_2 A_2 \quad \text{Equation (10b)}$$

The equation for the cross sectional area is shown below, where R is the radius of the needle.

$$A_1 = \pi R_1^2 \quad \text{Equation (11a)}$$

$$A_2 = \pi R_2^2 \quad \text{Equation (11b)}$$

Combining equations (10) and (11) and solving for the linear velocity gives equation (12) where d is the diameter of the needle.

$$u_1 = \frac{4Q_1}{\pi d_1^2} \quad \text{Equation (12a)}$$

$$u_2 = \frac{4Q_2}{\pi d_2^2} \quad \text{Equation (12b)}$$

Substituting equation (9) into equation (8) gives a new equation for matched momentum.

$$Q_1 \rho_1 u_1 = Q_2 \rho_2 u_2 \quad \text{Equation (13)}$$

Substituting equation (12) into the above and rearranging gives a new equation relating volumetric flowrate and densities to the ratio of the needle diameters.

$$\frac{Q_1^2 \rho_1}{Q_2^2 \rho_2} = \frac{d_1^2}{d_2^2} \quad \text{Equation (14)}$$

A simple rearrangement gives the equation for the volumetric flowrate of fluid 1.

$$Q_1 = Q_2 \frac{d_1}{d_2} \sqrt{\frac{\rho_2}{\rho_1}} \quad \text{Equation (15)}$$

If the diameters of the needles are the same for both fluids ($d_1 = d_2$) then equation (15) can be simplified.

$$Q_1 = Q_2 \sqrt{\frac{\rho_2}{\rho_1}} \quad \text{Equation (16)}$$

If fluid 1 is THF with a density of 881.4 kg/m³ at 25°C and fluid 2 is water with a density of 997.1 kg/m³ 25°C then,

$$Q_1 = 1.06 Q_2 \quad \text{Equation (17)}$$

However, as there are only two streams, in order to vary the weight percent of water in the mixer, fluid 2 has to be a mixture of THF and water.

To find the flowrate of the aqueous stream required for the matched momentum case, a series of simultaneous equations must be solved.

First the density of the fluid inside the mixer is found by:

$$\rho_{mix} = w_{H_2O} \rho_{H_2O} + (1 - w_{H_2O}) \rho_{THF} \quad \text{Equation (18)}$$

w_{H_2O} is the weight percent of water in the mixer and ρ_{H_2O} and ρ_{THF} are the densities of water and THF, respectively.

The total volume, V_{Total} of fluid that enters the mixer is the sum of the volume of the organic stream – containing the THF, magnetite and diblock – and the aqueous stream containing the water/THF mixture, $V_{organic}$ and $V_{aqueous}$.

$$V_{Total} = V_{organic} + V_{aqueous} \quad \text{Equation (19)}$$

The volume of the organic stream is pre-determined as the product of the volumetric flowrate and the time for which the experiment will be run.

$$V_{organic} = Q_1 t \quad \text{Equation (20)}$$

The volume of the aqueous solution is determined by:

$$V_{aqueous} = \frac{V_{organic}^2 \rho_{THF}}{m_{H_2O} + m_{THF(aq)}} \quad \text{Equation (21)}$$

m_{H_2O} is the mass of water in the mixer and $m_{THF(aq)}$ is the mass of THF in the aqueous stream.

m_{H_2O} is simply found by the product of the weight percent of water in the mixer, m_{mix} , and the total mass in the mixer as there is no water entering in the organic stream.

$$m_{H_2O} = w_{H_2O} m_{mix} \quad \text{Equation (22)}$$

$m_{THF(aq)}$ is found by subtracting the mass of THF in the mixer coming from the aqueous stream and m_{H_2O} from the total mass of fluid that enters the mixer m_{mix} .

$$m_{THF(aq)} = m_{mix} - m_{THF(org)} - m_{H_2O} \quad \text{Equation (23)}$$

The mass of fluid in the mixer is found by multiplying the density of the fluid in the mixer by the total volume.

$$m_{mix} = \rho_{mix} V_{Total} \quad \text{Equation (24a)}$$

Similarly, the mass of THF from the organic stream is found by the density of THF multiplied by the volume of the organic stream.

$$m_{THF} = \rho_{THF} V_{organic} \quad \text{Equation (24b)}$$

To find the volume of water and THF required for the aqueous stream the following equations were used:

$$V_{H_2O} = \frac{m_{H_2O}}{\rho_{H_2O}} \quad \text{Equation (25a)}$$

$$V_{THF(aq)} = V_{aqueous} - V_{H_2O} \quad \text{Equation (25b)}$$

Results and Discussion

Magnetite Nanoparticles

Determination of the water concentration at which

Figure 3:
magnetite-oleic acid (0.02mg/mL) versus PBO-magnetite (0.02mg/mL) clustering for determination of best magnetite particle for CIJ mixing trials.

Magnetite-oleic acid clusters before 1wt% water in THF but PBO-magnetite does not start clustering until 15 wt% water.

Magnetite-oleic acid clusters in the region of diblock micellization. However micellization occurs in the entire PBO-magnetite clustering region.

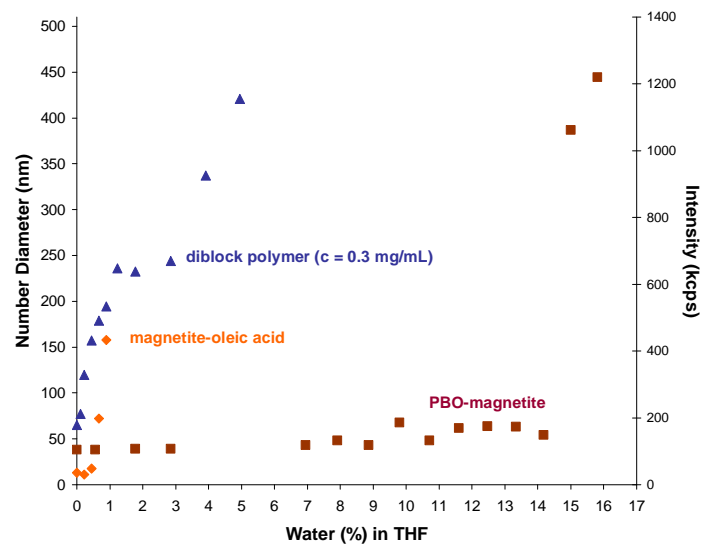
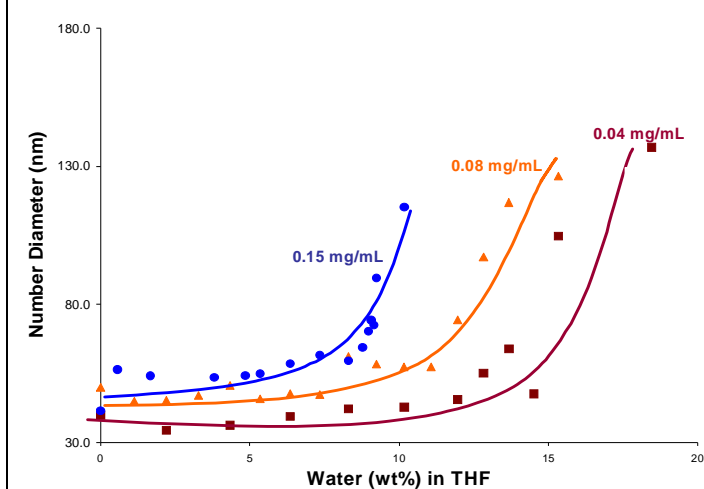
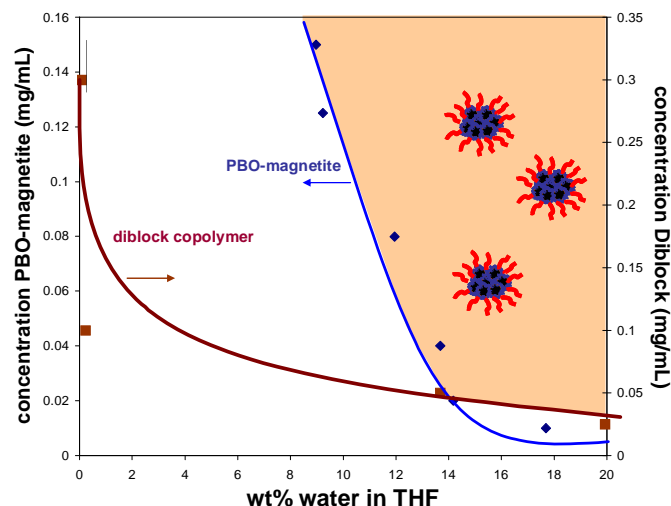


Figure 4: Critical Water Concentrations



A) Critical Water concentration for PBO-magnetite



B) Operating Region for CIJ mixer

both the hydrophobic magnetite particles clustered and the amphiphilic diblock copolymer micellized, both in THF, was essential to determining how to synthesize uniform, stable clusters. Figure 3 shows a plot of the change in cluster size of magnetite-oleic acid (0.02 mg/mL) and PBO-magnetite oleic acid (0.02 mg/mL) with increasing weight percent water in THF. Magnetite-oleic acid clusters appreciably between 0.04 and 0.07 weight percent water whereas PBO-magnetite clusters around 15 weight percent. If the clustering sizes of the magnetite particles are compared to a plot of the intensity of the diblock copolymer (0.3 mg/mL) versus weight percent water, it can be seen that micellization of the diblock copolymer occurs at a weight percent water similar to that at which clustering of the magnetite-oleic acid occurs. However, full micellization of the diblock copolymer occurs at water concentrations well below that needed for clustering of PBO-magnetite.

If magnetite-oleic acid is used for CIJ mixing experiments, difficulty would be expected in finding the optimal weight percent water needed for simultaneous clustering and micellization. However, by using PBO-magnetite, micellization can occur around single particles or clusters of particles, thereby eliminating concern for micellization kinetics.

It was also noticed that over time the magnetite oleic-acid would aggregate without the addition of water. Both suspension in THF as well as storage of

the sample under nitrogen were attempted. It was proposed that the oleic acid coating may be degrading over time with exposure to light, removing the steric stability and causing uncoated magnetite particles to aggregate.

No similar observations with stability of the PBO-magnetite were noted and thus many cluster formation experiments focused on this system.

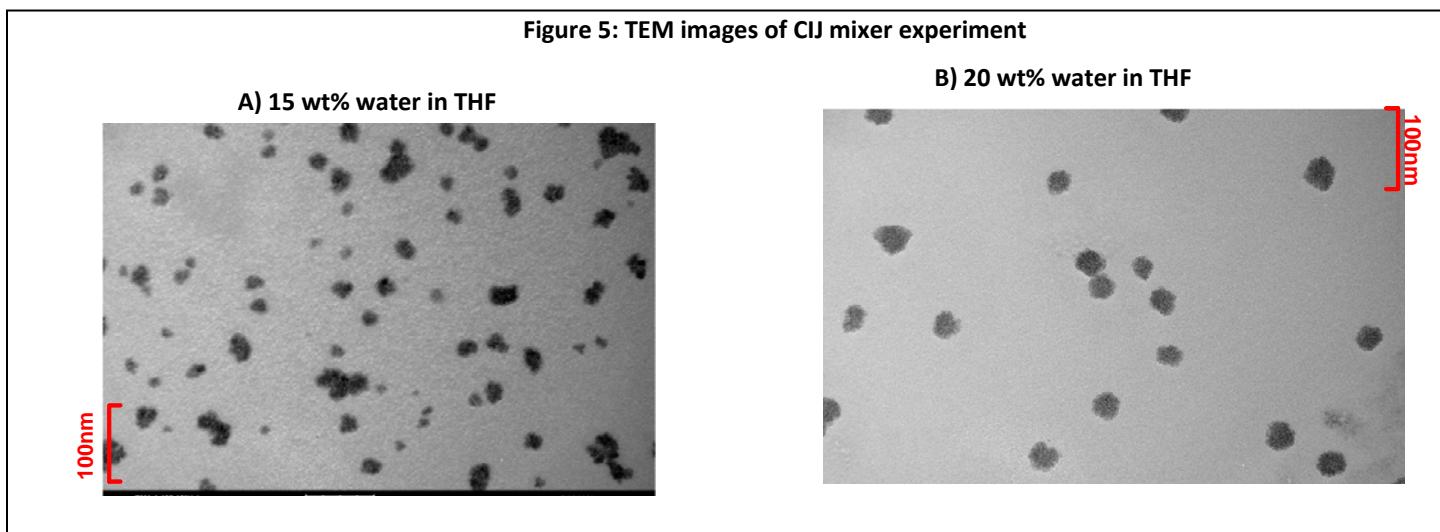
Critical Water Concentrations

Critical water concentrations were found for both PBO-magnetite and the Bz-PPO-*b*-PBO-OH diblock copolymer. Figure 4A shows the general trend that increasing weight percent water increases cluster size. Plotting the weight percent water required to form clusters or micelles at varying concentrations of the PBO-magnetite and the diblock copolymer gives a plot of operating conditions for the CIJ mixer as seen in Figure 4B. Operating the mixer to the right of the diblock

Table 1: Particle/cluster sizes for PBO-magnetite: PPO-*b*-PEO with varying wt% water

| wt% water | Q _{aqueous} (mL/min) | Dn (nm) |
|-----------|-------------------------------|---------|
| 5 | 39.7 | 39.6 |
| 10 | 39.5 | 32.6 |
| 15 | 39.2 | 161.3 |
| 20 | 39.0 | 207.0 |

Figure 5: TEM images of CIJ mixer experiment



copolymer line will result in micellization of the polymer. Operating to the right of the PBO-magnetite line would result in clustering of the particles. Operating the mixer in the shaded region should result in formation of the desired polymer stabilized magnetite clusters.

Using the CIJ Mixer

The CIJ mixer was operated within the operating region as illustrated in Figure 4A. The PBO-magnetite concentration was 0.02mg/mL and the diblock copolymer concentration was 0.03 mg/mL in the mixing chamber. The CIJ mixer was run at an organic flowrate of 40 mL/min for 30 seconds. The weight percent water in THF was varied from 5-20% at 5% increments. From Figure 3 it can be seen that clustering starts to occur at about 15 wt% water in THF. Table 1 shows the calculated aqueous flowrates and the cluster sizes measured by DLS. As expected, at 5 and 10 wt% water, there appear to be single particles with a micelle coating. At 15 and 20 wt% water, the number diameter (D_n) as measured by DLS is much larger, indicating that clustering may have occurred.

To verify the DLS measurements, transmission electron microscopy (TEM) was performed. The results for 15 and 20 wt% are shown in Figure 5 A and B, respectively.

Both images verify that clusters were formed but the most promising result is that, while at 15 wt% water the clusters are somewhat polydisperse, those clusters made at 20 wt% are fairly uniform.

Conclusions and Future Work

Polymer-stabilized magnetite clusters have successfully been synthesized using a confined impinging jet mixer. Using an operating region developed by the use of critical water concentrations for PBO-magnetite and Bz-PPO-*b*-PBO-OH, conditions for cluster formation were elucidated. Preliminary results appear to show that forming uniformly sized clusters may be possible.

Future work will include variation of PBO-magnetite and diblock copolymer concentrations as well as the weight percent water in THF to determine further effects of these components on cluster formation.

Experiments were also conducted at higher water concentrations but neither DLS nor TEM showed cluster formation. This may be due to the required mixing time not being achieved in the mixer. Mixing times should be calculated and flowrates should be adjusted accordingly if these experiments are to be repeated.

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