

# A GENERALIZED METHOD FOR MAGNETITE NANOPARTICLE STERIC STABILIZATION UTILIZING BLOCK COPOLYMERS CONTAINING CARBOXYLIC ACIDS

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**INTRODUCTION:** Magnetic nanoparticles that display high saturation magnetization and high magnetic susceptibility are of great interest for medical applications. Nanomagnetite is particularly desirable because it displays strong ferrimagnetic behavior, and is less sensitive to oxidation than magnetic transition metals such as cobalt, iron, and nickel. For *in-vivo* applications, it is important that well-defined organic coatings surround the nanomagnetite particles. It is rationalized that this will prevent any aggregation of the nanoparticles *in-vivo*, and may also enable efficient excretion and protection of the body from toxicity.

Magnetite nanoparticles can be prepared by coprecipitating iron (II) and iron (III) chloride salts in the presence of ammonium hydroxide at pH 9-10. Oleic acid is known to effectively stabilize dispersions of nanomagnetite in nonpolar solvents [1,2]. Stabilization occurs because the carboxylic acid group covalently reacts with the surface of the magnetite and the aliphatic chain extends out into the nonpolar solvent, preventing aggregation of the particles by a steric (entropic) mechanism. One goal of this work has been to develop a generalized methodology for stabilizing nanomagnetite dispersions using well-defined, non-toxic, block copolymers, so that the resultant magnetite-polymer complexes can be used in a range of biomedical materials. Our objectives have included: (1) Understanding what types of polymer structures bind irreversibly to magnetite at the physiological pH and what block lengths are desirable, (2) Tailoring polymer block lengths to maximize the concentration of bound magnetite, yet preserve good dispersion and (3) Designing copolymers with both hydrophilic and hydrophobic tail blocks to enable dispersion in different types of carrier fluids.

Triblock copolymers with controlled concentrations of carboxylic acids were designed as steric stabilizers for magnetite nanoparticle dispersions. The materials are comprised of hydrophilic, hydrophobic or amphoteric tail blocks to enable dispersion in media with various polarities, and central segments containing the carboxylic acid groups for anchoring to the nanomagnetite surfaces. A range of compositions involving poly(ethylene oxide) hydrophilic tail blocks have been investigated having averages of 3, 5, and 10 carboxylic acid-containing repeat units in the central, polyurethane anchor segment. Tail block lengths in this series have included 2000, 5000 and 15000 g/mol poly(ethylene oxide) oligomers which flank the central segments. Poly(dimethylsiloxane)

hydrophobic tail blocks and amphoteric poly(dimethylsiloxane)-b-poly(ethylloxazoline) tail blocks have been studied as a means for preparing hydrophobic dispersions. In all cases, the carboxylic acid groups were reacted into the central block to provide binding sites for the nanomagnetite particles (Figure 1). It is important that the carboxylic acid binding groups are closely located in the copolymer as opposed to being randomly distributed along the chains to afford the desired particle-polymer morphologies (Figure 2).

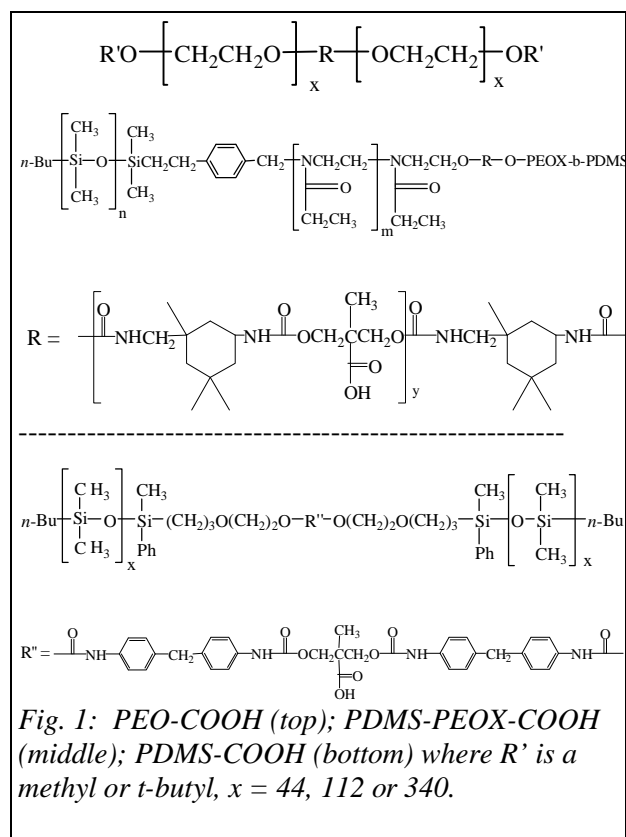


Fig. 1: PEO-COOH (top); PDMS-PEOX-COOH (middle); PDMS-COOH (bottom) where R' is a methyl or *t*-butyl,  $x = 44, 112$  or  $340$ .

**METHODS:** Sterically stabilized, neutral nanomagnetite dispersions were prepared by first forming the magnetite particles, then stabilizing the particle surfaces. A procedure for preparing a stabilized magnetite composition using a PEO-COOH triblock copolymer with 2000 g/mol  $M_n$  PEO endblocks and an average of three carboxylic acids in the central segment (2k-3-2k) is provided.

This composition utilizes 34.9 wt. %  $\text{Fe}_3\text{O}_4$  charged to the reaction. All solutions (water, base and polymer/ $\text{CH}_2\text{Cl}_2$ ) were carefully deoxygenated prior to use with ultrahigh purity dry nitrogen for a minimum of 30 minutes to avoid unwanted oxidation.

The first step of the reaction sequence involves forming magnetite nanoparticles in anaerobic conditions at ambient temperature. Aqueous solutions of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (0.389 M, 2.0 g) and  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (0.195 M, 0.736 g) reagents were prepared separately under  $\text{N}_2$  and syringed into a 3-necked, 250-mL, roundbottom flask equipped with a mechanical stirrer and pH electrode connected to a pH meter. The fittings for the apparatus were attached with vacuum-tight adapters to maintain an inert nitrogen environment.  $\text{NH}_4\text{OH}$  (50% v/v aqueous) was quickly syringed into the flask until a pH of 9.5 was reached (~10 mL) with rapid stirring (60 rpm) immediately after the aqueous iron salts addition. The solution quickly turned black indicating formation of the magnetite. The nucleation and growth of magnetite particles were allowed to occur for 30 minutes with stirring under a  $\text{N}_2$  atmosphere.

After this, the steric stabilizer solution comprised of the PEO-COOH copolymer dissolved in  $\text{CH}_2\text{Cl}_2$  (2 g polymer in 25 mL  $\text{CH}_2\text{Cl}_2$ ) was syringed into the flask and allowed to react with the magnetite for 30 minutes with stirring (pH ~ 8.5–9). The  $\text{CH}_2\text{Cl}_2$  solvent was subsequently removed with a strong  $\text{N}_2$  flow (~2 h) and the resulting polymer-magnetite nanoparticle aqueous suspension was neutralized with dilute HCl (25 % v/v aqueous) to a pH ~ 6.5–7.

The resulting stable dispersion was transferred to a dialysis membrane (Spectra pore 7, MWCO 1000) and dialyzed against water for three days, refreshing the dialysis water twice/day. Particle aggregates in the salt-free magnetite ferrofluids were removed by centrifuging for 30-minute time intervals where the sediment was discarded and the process was repeated until no precipitation was observed in the bottom of the centrifuge tube. This generally required centrifuging the magnetite for 3 – 5 intervals.

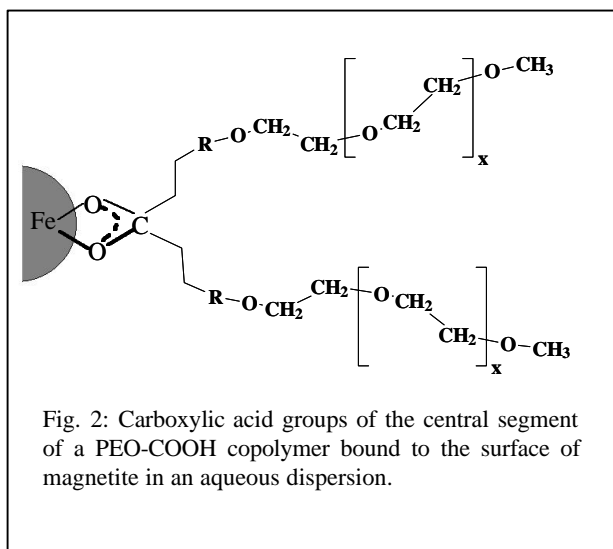
The method for preparing magnetite dispersions using the nonpolar stabilizers, such as PDMS-COOH or PDMS-PEOX-COOH, is similar. Interfacial interactions allow for the magnetite to be extracted into the organic phase in these dispersions.

## RESULTS AND DISCUSSION:

**Synthesis of magnetite dispersions.** Magnetite nanoparticles were synthesized by co-precipitating aqueous  $\text{FeCl}_2$  and  $\text{FeCl}_3$  salt solutions at room temperature under  $\text{N}_2$  with a hydroxide base. The stoichiometric molar ratio of  $\text{Fe}^{2+}/\text{Fe}^{3+}$  was 0.5 to achieve quantitative conversion. All solutions were deoxygenated prior to use and reacted immediately to minimize oxidation of the  $\text{Fe}^{2+}$  species [3,4]. The two iron salts were dissolved separately and combined with vigorous mixing, just prior to base addition, and the base was quickly syringed into the flask with rapid stirring (~ 1–3 s) to a pH of 9.5–10. This produced a stable nanomagnetite dispersion in water, electrostatically stabilized with ammonium ion double layers.

Two slightly different methods for coating the nanomagnetite with the polymer stabilizers were used, depending on the solubility of the polymers in water. Numerous groups have previously reported that if carboxylic acid functional groups are present in the iron oxide solution during magnetite synthesis, the crystallization process to form the cubic inverse spinel crystalline structure of magnetite is greatly inhibited, and magnetite does not form [5,6].

The copolymer stabilizers which contained the hydrophilic poly(ethylene oxide) tail blocks were soluble in the aqueous iron salt solutions, so these copolymers were not added until after the magnetite nanoparticles had formed. The polymers were introduced to the aqueous magnetite particle dispersions as solutions in dichloromethane, a step which produced two-phase mixtures. Since these copolymers are soluble in both the aqueous and organic phases in this case, they partition between the two phases. It is not yet clear whether the stabilizer must be located within the aqueous phase to efficiently react with the particles, or whether a substantial portion of the coating reaction can take place at the water-solvent interface, and it is recommended that further attention be given to this issue as the work progresses. Following reaction of the magnetite-polymer complex for 30 minutes in the two-phase mixture, the dichloromethane was removed with a strong  $\text{N}_2$  purge to transfer all of the polyethylene oxide based stabilizer into the water phase. It should be noted that if stirring is stopped at this stage, the magnetite settles out of solution. This suggests that the carboxylate groups are not yet irreversibly bound to the surface of the magnetite. The pH of the reaction mixture was subsequently lowered to pH 6.5 – 7 by titrating with acid, whereupon stable dispersions were obtained.



The hydrophobic and amphoteric stabilizers which contained poly(dimethylsiloxane) tail blocks were insoluble in the aqueous iron salt solutions. Hence, these polymers were added to the aqueous iron salt solutions in dichloromethane to produce a two-phase mixture before magnetite was formed. Subsequently, the base was added to the multiphase reaction to crystallize and precipitate the nanomagnetite in the aqueous phase (while the polymer was present in the organic phase). This process results in the coated magnetite being extracted into the organic phase. Subsequently, the mixtures were neutralized and the organic phase was dialyzed and centrifuged to remove any minor concentrations of aggregates that may have formed. Again, the locus of coating reaction is unknown and warrants further study. Interestingly, only the amphoteric copolymers with the hydrophobic poly(dimethylsiloxane) block connected to a hydrophilic poly(ethylloxazoline) block were able to efficiently “reach” into the aqueous phase and coat the magnetite. Table 1 shows clearly that if the hydrophilic block is not present (e.g., PDMS-COOH in table 1), only minimal concentrations of magnetite become coated.

Aqueous dispersion stabilities of the magnetite nanoparticles coated with the hydrophilic poly(ethylene oxide) containing block copolymers were investigated as a function of pH. Results suggest that the magnetite coating process should be conducted at pH of 8-10, then the dispersions should be neutralized. Stable dispersions were observed only at pH 7 and below. At neutral and lower pH, the carboxylate group chemisorbs onto the surface of magnetite, and sterically stabilizes the dispersions (figure 2). This provides stable dispersions at physiological pH's which are not sensitive to ionic strength or pH changes. Transmission electron photomicrographs of all of the samples show magnetite particles ~10 nm in diameter. Magnetite crystalline structure was confirmed by powder X-ray diffraction and electron diffraction. The magnetite nanoparticles appear to be single crystals.

Magnetic properties of these polymer-magnetite nanoparticle systems were investigated by vibrating sample magnetometry in the solid state. The saturation magnetization was determined from the

plateau region of the magnetization displayed by the sample in the presence of an externally applied field varied from 8000 Oe to -8000 Oe. The magnetization data was quantified in terms of magnetite using elemental analysis to determine the concentration of iron in the materials (Table 1).

Table 1. Magnetic properties of polymer stabilized magnetite nanoparticles.

Sample	Conc. Fe <sub>3</sub> O <sub>4</sub> charged (wt. %)	Conc. Fe <sub>3</sub> O <sub>4</sub> obtained (wt. %)	M <sub>s</sub> /g sample (emu/g)	M <sub>s</sub> /g Fe <sub>3</sub> O <sub>4</sub> (emu/g)
2k PEO-COOH	35	23	17	74
5k PEO-COOH	46	30	20	66
PDMS- <i>b</i> -PEOX-COOH	30	26	17	68
PDMS-COOH	30	3.9	1.6	40

Experimental saturation magnetization values for magnetite nanoparticles reported in the literature range from 30–60 emu/g, whereas bulk magnetite can theoretically be as high as 92 emu/g [7,8]. Thus, the response of the magnetite nanoparticles described herein compare favorably with previous materials. The lower magnetizations for the nanoscale particles have been attributed to surface effects.

The concentration of magnetite in these materials has not yet been maximized, and TEM photomicrographs suggest there are significant amounts of excess polymer remaining in the samples. It will be necessary to eliminate the excess polymer to achieve higher magnetite concentrations, and hence higher M<sub>s</sub> per gram of sample. It is clear that the minimum tail block length investigated thus far can be reduced further, which should enable the concentration of magnetite relative to polymer to be raised further.

**CONCLUSIONS:** The magnetite is formed first by aqueous coprecipitation of di- and trivalent iron salt solutions in a nitrogen environment and stabilized afterwards. The magnetite particles formed are ~10 nm in diameter, independent of the polymer stabilizer used because they were formed prior to stabilization. Stable dispersions were achieved at neutral pHs with all of the hydrophilic poly(ethylene oxide) based polymer stabilizers. Powder X-ray diffraction patterns of these systems suggest the magnetite crystalline structure for all samples.

The saturation magnetization of these ~10 nm polymer-coated particles are ~66–74 emu/g Fe<sub>3</sub>O<sub>4</sub>. Such behavior is high for magnetite nanoparticles, and suggest “good quality” magnetite. However, the maximum concentration of magnetite achieved thus far results in a saturation magnetization of only ~20 emu/g, and TEM suggests that there is a significant excess of polymer remaining. Thus, one key aspect of our continuing work will be to

maximize the magnetite/polymer concentration, yet maintain stable dispersions.

Nanomagnetite stabilization using the nonpolar PDMS-COOH and PDMS-*b*-PEOX-COOH stabilizers provided significantly different results. The PDMS-*b*-PEOX-COOH stabilizer was able to incorporate more than ten times more magnetite per gram than the PDMS-COOH stabilizer. It is proposed that the hydrophilic PEOX block facilitates the reaction with the magnetite surface. The presence of the hydrophilic PEOX block adjacent to the carboxylic acid groups makes the carboxylic acid groups more available for reaction with the magnetite in the aqueous phase.

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