Characterization of Iron Oxide Integration within Phospholipid Encapsulated Colloids

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Abstract and Introduction:

Contrast agents for magnetic resonance imaging (MRI) enhance the visibility of targeted tissues and cells. Many current T_1-weighted (T_1w) contrast agents (bright) are gadolinium-based; however, gadolinium has been associated with nephrogenic systemic fibrosis, a severe connective tissue disease, among patients with impaired kidney function. Iron oxides are common non-gadolinium nanoparticles used for dark MRI imaging (T_2*-weighted). They provide bold negative contrast due to their strong magnetic field interference, but their prolonged duration in the blood stream precludes imaging for 24 to 48 hours post-injection, until the circulating metal oxide concentration has decreased sufficiently.

Colloidal iron oxide nanoparticles (CION) also utilize iron oxide crystals, but uniquely produce bright T_1w contrast and allow imaging within an hour. The novelty arises from the chemical coupling of the magnetite particles within the inner aspect of a monolipid layer, which distributes the metal predominantly around the perimeter. By avoiding the clustering of iron within the core of the particle and using magnetite, a weaker form of iron oxide, T_2* effects are reduced. While chemical cross-linking of the iron oxide particles with the lipid membrane after the formation of the particle has been effective, a new chemical formulation to achieve a self-assembly product is preferable. This project examined the utility of transmission electron microscopy (TEM) to characterize the spontaneous distribution of lipid-modified iron oxides into the hydrophobic surfactant of CION for development of a new chemical synthesis.

To further evaluate the model suggested by the CION particle chemistry, a particle in which the metal was oriented to the periphery and excluded from the center by an acrylamide hydrogel core was synthesized. The goal was to determine if the T_1/T_2* ratio of these particles was improved.

Experimental Procedure:

A modification of our previously published formulation for synthesizing the CION particles was followed (Figure 1) [1]. Cholesterol and undecenoyl chloride were heated to reflux in pyridine and the product was recrystallized from ethanol. The modified cholesterol was then cross-linked to oleate-coated magnetite to covalently anchor the iron oxide particles to the membrane.

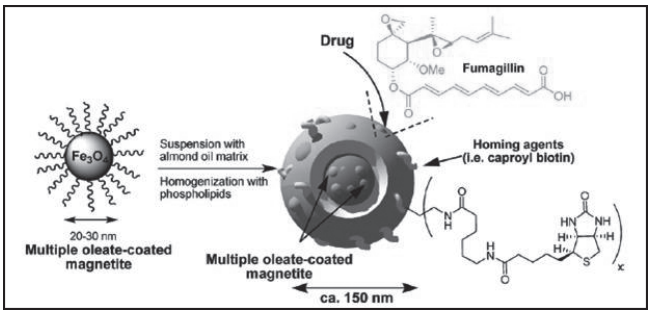

Figure 1: Schema of production of CION particles.

CION samples were prepared for transmission electron microscopy (TEM) as follows: particles were fixed with 2.5% glutaraldehyde in phosphate buffered saline for 30 minutes, and centrifuged into a pellet. After post-fixing with 1% osmium tetroxide, 2% tannic acid, and 4% uranyl acetate, the particles were embedded in EMBED-812, sectioned, and imaged with TEM at 120 kV.

To prepare the hydrogel emulsion, a 5:1 molar ratio of lecithin:cholesterol was dissolved in chloroform, which was evaporated to make a thin lipid film. This film was hydrated by a mixture of 4.5% acrylamide, 0.5% *N,N'*-methylenebis(acrylamide), and 0.0012% Irgacure 2959 (a photoinitiator) in HEPES buffered saline [2]. The mixture was sonicated and hand-extruded through a syringe using successive filters of 1 and 0.2 μm pore size to reduce aggregates. Ascorbic acid was used to prevent peripheral cross-linking of the particles. The mixture was placed in a UV cross-linker for five minutes, and the experiment was repeated several times with manganese oleate incorporated into the lipid film to 30% and 50% by weight.

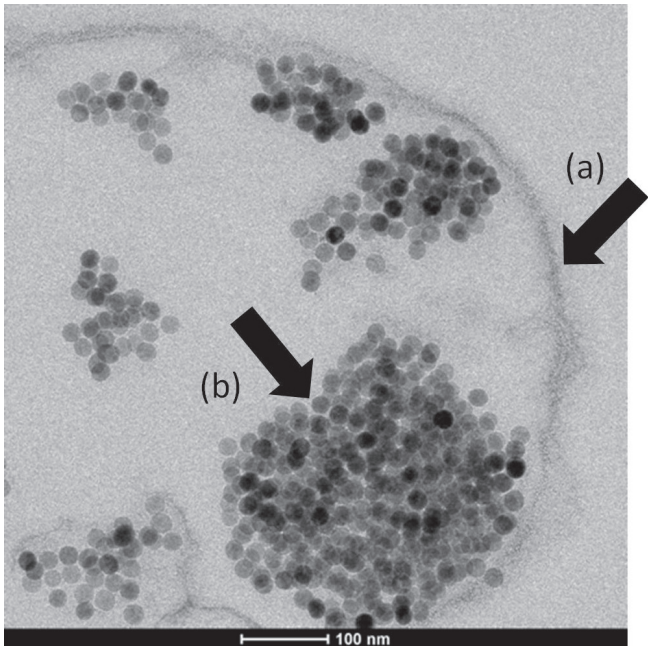

Figure 2: TEM of CION particles. (a) Lipid membrane ruptured during centrifugation. (b) Aggregation of iron oxide particles.

Results and Conclusions:

Size Determination. The hydrodynamic diameter and zeta potential of the CION particles were measured using dynamic light scattering and found to be 141.0 ± 0.3 nm and -27.32 ± 0.64 mV, respectively.

Transmission Electron Microscopy. TEM was used to examine the distribution of iron oxides within the membrane. Fixation of CION proved to be complex, as the images obtained show a ruptured lipid layer with evidence of aggregation (Figure 2). Review of the process suggested that the centrifugation step likely contributed to the particle breakdown. Work on a revised TEM processing scheme is continuing.

Magnetic Resonance Characterization. The MR relaxivity of successive dilutions was measured (Figure 3). The T_1 relaxivity of CION was found to be 0.1482 $([Fe]mM \cdot s)^{-1}$, a very poor relaxivity. This result suggests that the anchoring of iron oxides to the periphery of the membrane was too weak, allowing the metal to aggregate in the core within the strong magnetic field of the MRI.

Hydrogel. The size of the hydrogel particles was determined using dynamic light scattering. The hydrodynamic diameter of the particles without manganese oleate was found to be 442.0 ± 5.7 nm, and the diameter with manganese oleate incorporated to 30% was found to be 443.9 ± 12.4 nm, indicating that incorporating the manganese oleate was successful. Loading the manganese oleate at 50% was unsuccessful, leading to aggregation. Multiple populations were evident in the data (Figure 4), which indicates that the syringe extrusion process was not adequate, perhaps causing the large particle sizes.

Future Work:

Research will continue to improve the CION chemical coupling to achieve the proper morphology in a self-assembly synthesis without added post-crosslinking reagents.

Additionally, work on the hydrogels to study the physics of the metal distribution in nanoemulsions will continue.

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References:

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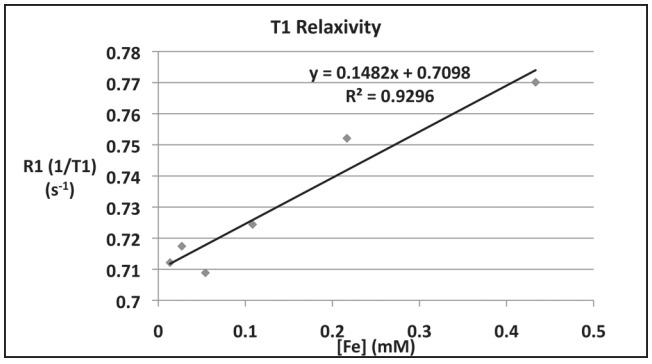

Figure 3: Graph of T_1 relaxivity versus iron concentration.

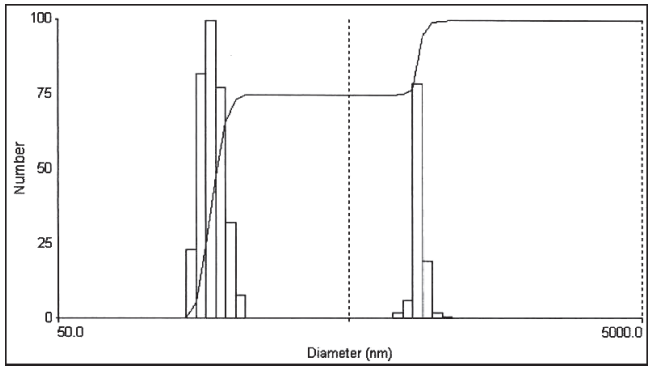

Figure 4: Distribution of particle sizes measured by dynamic light scattering.