Synthesis, Characterization and Application of Citrate Modified Superparamagetic Iron Oxide Nanoaparticles as a New Contrast Agent for Magnetic Resonance Imaging

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Introduction: The endogenous contrast in magnetic Resonance Imaging (MRI) depends on the chemical and physical nature of tissues and often arises from local variation in proton density. The use of contrast agent, which alters the signal intensity by selectively shortening the hydrogen relaxation time of the tissue, becomes essential to improve sensitivity and specificity of MRI (1, 2). The conventional, paramagnetic gadolinium chalets typically behave as T1 contrast agent and provide hyperintensity in image where they accumulate. On the other hand, superparamagnetic nanoaprticles behave as T2 contrast agent, thus show hypointensity at accumulated points. Most of these contrast agents are based on superparamagnetic properties. Unlike ferromagnetic materials, superparamagnetic nanopartilces show high magnetization and zero coercivity; thereby making this material useful for biological applications. The classifications of these are based on their size: superparamagnetic iron oxide (SPIO) and ultra small superparamagnetic iron oxide (USPIO) (3). Surface fuctionalization has strong effect on the performance of magnetic nanopartilces in its contrast applications. The particles are often designed to be target specific through coating with polymers (Dextran, functionalized polyethylene glycol (PEG) etc.), liposoms, proteins, etc. Citrate coated iron oxide nanopartilce is a novel USPIO contrast medium for application in MRI.

The study aimed to develop a new chemical synthesis method of citrate coated iron oxide nanopartilces which show good magnetic properties, monodispersive and its dispersion stability and to look for their behavior in biological systems that may eventually lead to its development as an MR contrast agent.

Materials and Methods: In the current study, synthesis of citrate coated Fe_3O_4 magnetic nanopartilces was done by high temperature co precipitation method. In this process, $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ was taken in 2:1 ratio in high boiling solvent Diethyle Glycol (DEG) and ammonia were used as a base. This reaction mixture was heated at 150 ^{0}C in argon atmosphere. After 30 min at this temperature, calculated amount of citric acid was added to the reaction mixture. The temperature of solution was increased to 180-190 ^{0}C and kept it constant for 2 h. After completion of reaction, solution was cooled and washed with acetone and kept for drying. The characterizations of so formed product were carried out by powder X-ray diffraction (XRD), Fourier Transformed Infrared (FTIR), Transmission Electron Microscopy (TEM). Magnetic measurement was performed by Vibration Sample Magnetometer (VSM). Dispersion of different concentrations was made in saline water. To see the cellular uptake of magnetic nanoparticles, mouse macrophage cells (RAW 264.7) and Jurket cell line (clone E6-1) which is a T-cell line (lymphocytic origin) that has non adherent property (floating in nature) were grown in RPMI-1640 supplemented with 10% heat inactivated fetal calf serum, 50U/ml penicillin, 40 mg/ml streptomycin and 0.3mg/ml L-glutamine. Cells (2×10^6) were incubated in a 5% CO_2 with filter-sterilized suspension of magnetic nanoparticles for different incubation times (24 to 72 h) and various extra cellular iron concentrations (0.03 mg/ml-0.8 mg/ml). The cells which showed uptake of magnetic nanoparticles, confirmed on Prussian blue staining were subjected for MRI. T2, T1, and T2* gradient recalled echo sequence (GRE) images were obtained on a 1.5 T GE scanner.

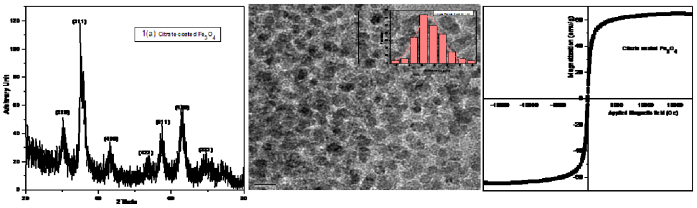

Fig 1: a) Powder x-ray pattern, **b)** Transmission electron microscopy (TEM) image, **c)** Magnetization curve of citrate coated Fe_3O_4 nanopartilces

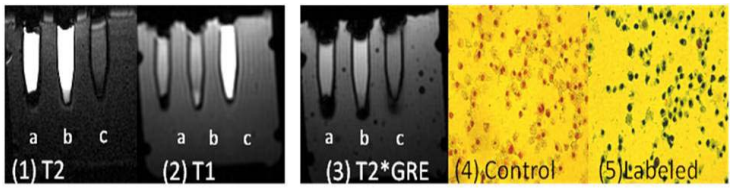

Fig 2: 1-3; MR images of tubes containing **a)** labeled cells, **b)** nonlabeled (control) cells **c)** nanoparticle solution; 4- nonlabeled cells (RAW) showing negative Prussian blue staining; 5- labeled cells (RAW) showing positive Prussian blue staining

Results and Discussion: In this study we synthesized citrate coated Fe_3O_4 magnetic nanopartilces by high temperature co precipitation method using Citrate as a coating agent in DEG. DEG plays an important role in controlling the particle size by coordination to metal ions. The phase characterization and size of prepared Fe_3O_4 are confirmed by Powder x-ray diffraction (Fig 1(a)) and transmission electron microscopy. The Crystallite size of material is calculated by using Scherrer formula and it is found to be 6±1 nm. The particle size calculated from TEM image (Fig 1(b)) is 6±1 nm and it is monodispersive in nature. FTIR study shows the interaction of citric acid on the surface of iron oxide nanoparticles. From the FTIR study, it is confirmed that carbonyl groups of citric acid show two band at 1639 and 1454 cm^{-1} instead of 1730 cm^{-1} , which indicates the covalent attachment of acid carbonyl groups on iron oxide surface. The magnetic study of citrate coated iron oxide is performed and it shows superparamagnetic nature with zero coecivity and high saturation magnetization of 65 emu/g, which is very close to bulk material (86emu/g). The dispersion of citrate coated iron oxide is very stable and no precipitation is observed. We observed that 0.4mg/ml of extracellular iron concentration result in more than 95% of cellular uptake in RAW cell line and 20-30% uptake in jurket cell line and cells of both RAW and jurket cell lines were perfectly intact upto 96 hours of incubation. Less uptake of magnetic nanoparticles in jurket cell line may be due to the fact that jurket cell line is not phagocytic but the uptake may indicate the nonspecific engulfment of these nanoparticles like that of dendimers (6) On T2*GRE sequence the labeled cells showed strong T2* effect. On proper optimization these nanoparticles may be used as MR contrast agents to locate the labeled cells into the whole body and analysis of their trafficking dynamics and localization. It differs from other SPIO or USPIO compounds currently under development or in clinical use and coated with polymers such as dextran, carboxydextran, starch, or polyethylene glycol in that the particles are stabilized with the monomer citrate. We conclude that this so-called electrostatic stabilization allows the production of particles with an overall total diameter that is even much smaller than that of conventional USPIO particle (5, 6).

References**:** 1- Martina, et al. J. Am. Cm. Soc 2005;127;10676-10685, 2- Younan, et al. Adv. Mater. 2007; *19*; 33-60, 3 - Corot, et al. Ad. Drug Del. Rev; 2006;58;1471-1504, 4- Bulte, et al. J Cereb Blood Flow Meta.; 2002; 22, 899-907 5- Shen ,et al. Magn Reson Med.; 1993; 29:599–604, 6- *Matthias, et al.* Inv. Radio; 2004; 39; 394-405,