

## Recent Progress on the Preparation of Magnetic Iron Oxide Nanoparticles for Bio-Imaging Applications

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Magnetic Resonance Images present excellent spatial resolution, but relatively low sensitivity. In order to improve it the use of contrast agents (CA) is strongly required. Magnetic iron oxide nanoparticles are one of the few FDA approved for this purpose. Commercial CA of this group have aggregate sizes in dispersion  $\geq 50$  nm, and are cleared from the blood flow and accumulated in the liver and spleen in short times. This issue impairs the clinical application of CA-enhanced MRI to the early detection of cancer or inflammatory lesions in regions other than the liver.

The BONSAI Project aims at the development of stable colloids of non-toxic, non-immunogenic NPs with: (i) high magnetization (ii) particle size small enough and adapted coating to remain longer in circulation after injection (“stealth NPs”).

The NP synthesis procedure employed by the BONSAI partners is a one-step process based on laser pyrolysis of suitable gas-phase precursors. Using this method in continuous operation mode, the BONSAI partners succeeded in the production of samples of 10 g of iron oxide powders, with small particle size (1.9-3.6 nm), narrow size distribution and good magnetic properties.

Applications in biology require that MNPs are well dispersed and stable in physiological media, and great effort was devoted to the preparation of stable colloidal solutions of MNPs. In order to achieve this goal, the MNP's were dispersed in water + citrate or alternatively in hexane/toluene + oleic acid oleylamine with no alteration of their magnetic properties. The water-citrate samples reached the USPIO level ( $D < 40$  nm) after the removal of aggregates, and presented good stability at high concentrations ( $>5$ mg Fe/ml). The organic-oleate approach produced colloids with  $D < 200$  nm. Both of them are able to be redispersed after drying with little change in the aggregate size distribution. Alternative further coatings have been developed in order to make the colloids stable and biocompatible in the body. In the BONSAI project the citrate of the water-citrate colloids was exchanged by L-Dihydroxyphenylalanine (L-dopa). This compound was chosen due to the affinity of its catechol group towards the iron oxide and its non toxic nature. The L-dopa coated nanoparticles are stable at physiological salt concentration of 150mM NaCl, and stable in culture medium. The use of polymeric coatings for the same purpose is under study using the ATRP approach on the hydrophobized oleylamine modified NP's. The warm microemulsion process developed by NANOVECTOR was used to directly encapsulate project NPs in Solid Lipid Nanoparticles (SLNs). By these coating the colloidal stability was much improved without alteration of the particle size distribution, being remarkable that the process SLNs loading disaggregate the colloid with much smaller final hydrodynamic sizes ( $< 80$  nm - Intensity mean)

Bio-testing is a major issue in the BONSAI project. These tests, still in progress, include the study of the uptake of the MNP in epithelial and PMA-activated macrophage-like THP-1 cells and other cell lines and various toxicity tests (apoptosis, oxidative stress, eosin exclusion test, LDH and MTT assays on A30 alveolar epithelial cells and L929 mouse fibroblasts).