

## ***In vitro* toxicity studies of polymer coated superparamagnetic iron oxide nanoparticles**

Lamiaa M.A.Ali<sup>1,2</sup>, **Víctor Sorribas**<sup>2\*</sup>, Rafael Piñol<sup>1</sup>, Lierni Gabilondo<sup>1</sup>, Angel Millán<sup>1</sup>,  
Fernando Palacio<sup>1\*</sup>

<sup>1</sup> Instituto de Ciencia de Materiales de Aragón. CSIC - Universidad de Zaragoza, Spain. E-mail: [palacio@unizar.es](mailto:palacio@unizar.es)

<sup>2</sup> Laboratory of Molecular Toxicology, Universidad de Zaragoza, Spain. E-mail: [sorribas@unizar.es](mailto:sorribas@unizar.es)

Superparamagnetic iron oxide nanoparticles (SPIONs) are inorganic nanomaterials involved in many biological and medical applications, e.g., in diagnosis as MRI contrast agent or in therapy as an agent in hyperthermia treatments. Our model consists of ferric oxide nanoparticles embedded within poly(vinylpyridine) (P4VP) and coated with polyethylene glycol (PEG). A fraction of coating PEG can also be functionalized for the conjugation of fluorescent dyes, antibodies and drugs. The particles are dispersed in phosphate buffer saline (PBS) at pH 7.4 to mimic physiological conditions. The resulting ferrofluids have core diameter (ferric oxide nanoparticles diameter) ranging between 4 to 15 nm, with 10% size dispersion, and hydrodynamic diameter ranging between 50 to 164 nm.

Cytotoxicity studies of the ferrofluids have been carried out in two different cell lines [1], opossum kidney cells (OK) and vascular smooth muscle cells (VSMS). The activity of the lactate dehydrogenase in culture media was determined as a function of the dose. LC50 has been calculated and the toxic effect was due to accumulative effects with time. Ethidium bromide/acridine orange tests with the help of the fluorescent microscope show that the cell death is due to necrosis rather to apoptosis. These results are confirmed by DNA fragmentation test. No oxidative stress findings have been observed. The studies have been extended to determine the cytotoxicity effects of the magnetic core particles as a function of their size. The results show that cytotoxicity increases as the diameter of the nanoparticles decreases.

Sub cellular tracking studies have been carried out using fluorescent nanoparticles. The results show the localization of the nanoparticles after 24h of incubation with the cells inside the endolysosomal system, as shown in the Figure. Kinetic studies show the internalization of the nanoparticles inside the cells after 4h of incubation, increasing with time until 12 h and then decreasing. Nanoparticles uptake takes place by clathrin-dependent endocytosis and the rate of internalization depends on cell line and nanoparticles size.

[1] R. Villa-Bellosta, G. Ibarz, A. Millan, R. Piñol, A. Ferrer-Dufol, F. Palacio, V. Sorribas. *Toxicol. Lett.*, **180**, S221, (2008).

