PROTEIN-CARBOHYDRATE INTERACTION STUDIES BY MEANS OF SUPERPARAMAGNETIC NANOPARTICLES CLUSTERING.

María Moros, Beatriz Pelaz, Carlos Cuestas, Valeria Grazú, Jesús M. de la Fuente
Instituto Universitario de Investigación en Nanociencia de Aragón (INA), University of Zaragoza, C/ Cerbuna 12, 50009-Zaragoza, Spain
mamoros@unizar.es

Magnetic nanoparticles offer a wide range of new opportunities including the quality improvement of contrast agents for MRI, hyperthermic treatment, and site-specific drug delivery. All these biological applications of these nanoparticles require the fulfilment of several features: high magnetization values, size smaller than 20 nm, narrow particle size distribution, simple biofunctionalization, a special surface coating to prevent nanoparticles aggregation, opsonization and toxicity effects [1]. Very recently, Sun and co-workers [2] had markedly improved the synthesis of monodispersed magnetite particles with size around 4 nm by thermal decomposition of iron (III) acetylacetonate in phenyl ether in the presence of oleic acid and oleylamine. As these nanoparticles are coated with a hydrophobic organic layer, they are only stable in hexane and other non-polar or weakly polar organic solvent. We report the optimization of a procedure to prepare magnetite nanoparticles with an average size of 8 nm, a narrow size distribution and high stability in water and physiological media. The procedure to stabilize the nanoparticles in physiological media is based on a previously reported strategy [3], which takes advantage of the hydrophobic surfactant layer of these nanoparticles to introduce an amphiphilic polymer shell. On top of that we propose these nanoparticles as good candidates for further applications in molecular imaging and biosensing.

It is well known that superparamagnetic iron oxide nanoparticles could be used as transversal relaxation (T_2) MRI contrast agents [4]. The superparamagnetic behaviour of our nanoparticles (Nps) suggested that they could be an efficient T_2 relaxation agent. ^{1}H NMR relaxation times studies were performed using a 1.5 Tesla in a Bruker Minispec NMR spectrometer. The Nps showed exceptionally high r_2 relaxivity values which clearly demonstrate the potential of our Nps as a T_2 MRI contrast agent.

Taking advantage of their T_2 relaxation times, these nanoparticles were also used to evaluate carbohydrate-protein interactions. The low affinity of the biological interactions where carbohydrates are involved makes very difficult the study of these kinds of interactions. Nps are good platforms to be used in these studies, as could compensate the low affinity of the interactions by multivalent presentation of the ligands [5]. Moreover, it had been already described that clustering strongly affects the transverse (T_2) relaxation times induced by superparamagnetic Nps [6]. Therefore by coupling the presence of carbohydrate binding proteins to the induction of aggregates between the corresponding ligand-functionalized superparamagnetic nanoparticles, very sensitive aggregation-based sensors could be designed in order to detect such extreme low affinity interactions. To perform these studies we have selected concanavalin A (Con A) as model of carbohydrate binding protein. This plant lectin binds specifically to certain structures found in various sugars, namely internal and non-reducing terminal mannosyl or glucosyl residues [7]. A range of concentrations of Con A was added to a solution of glucose-functionalized Nps, and T_2 relaxation times were recorded. The results showed changes in T_2 relaxations, even using extreme low concentrations of ConA. If enough glucose was added the Np aggregates were undone, as the free glucose could bind to the active sites of Concanavalin A, which makes the system reversible (Figure 1). Moreover, the high specificity of the system was proved as no changes in T_2 value were observed when using galactose-functionalized Nps. All these results make us think that very sensitive aggregation-based sensors of carbohydrate binding proteins could be designed using these Nps functionalized with the corresponding ligand.
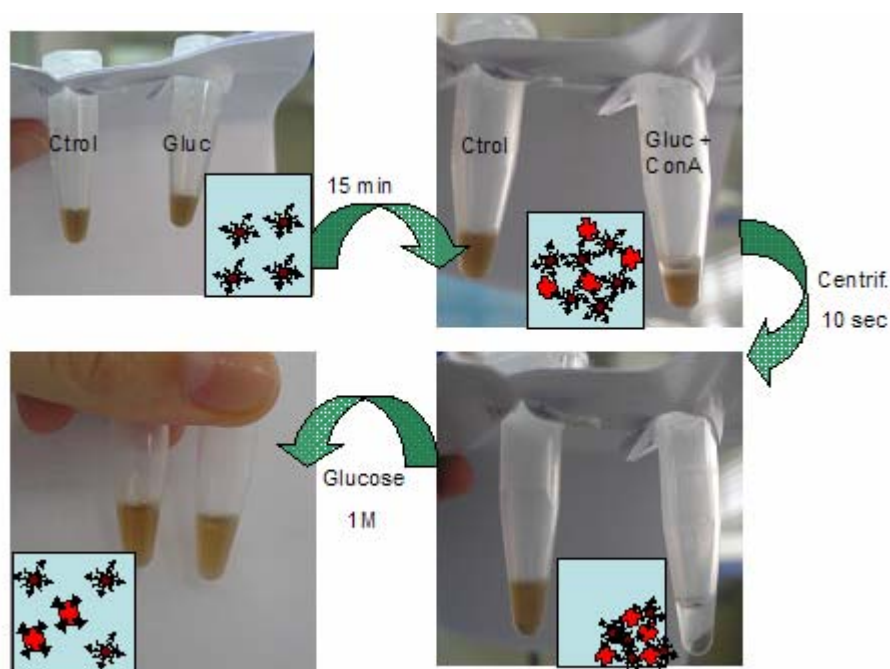
Figure 1. Aggregation of glucose-functionalized superparamagnetic nanoparticles due to the presence of Concanavalin A.

References:
[1] Berry C.C. and Curtis A.S.G. *J. Phys. D: Appl. Phys.* **2003**, 36, R198-R206.
[2] Sun S., Zeng H., Robinson D.B., Raoux S., Rice P.M., Wang S.X. and Li, G. *J. Am. Chem. Soc.* **2004**, 126, 273-279.
[3] Pellegrino T., Manna L., Kudera S., Liedl T., Kokysh D., Rogach A.L., Keller S., Rädler J., Natile, G. and Parak, W.J. *Nanoletters* **2004**, 4, 703-707.
[4] Sun C., Lee J.S.H and Zhang M., *Adv. Drug Deliv* **2008**, Rev. 60, 1252-1265.
[5] De la Fuente J.M. and Penadés S. *Biochimica et Biophysica Acta* **2006**, 1760, 630-651.
[6] Sosnovik D.E. and Weissleder R. *Curr Opin Biotechnol* **2007**, 18, 4-10.
[7] Smith E. and Goldstein I. *Arch Biochem Biophys* **1967**, 121, 88, 1967.