Synthesis and physico-chemical characterization of iron nanopartiles grafted by vectorizing molecules

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INTRODUCTION:

One of the main challenges in medicine is to develop efficient and reliable tools for early and specific diagnosis of pathologies, among which cancers. One way is the use of nanomagnets grafted with molecules specifically targeting one kind of pathological cells. Superparamagnetic particles are used as contrast agents allowing for the detection of very small tumors by Magnetic Resonance Imaging (MRI) through their property to modify locally the signal intensity of the images. This contrast enhancement is due to stimulation of the water proton nuclear relaxation rates. The grafting of vectorizing molecules on the coating of these particles could affect their magnetic properties, hence their efficiency. The aim of the present work is to evaluate the effect of the binding of some vectors on the relaxometric properties of the superparamagnetic particles.

MATERIAL AND METHODS:

Colloidal nanomagnets coated with dextran are obtained by coprecipitation of a solution of ferric and ferrous ions with ammonia in the presence of dextran. The target molecules are covalently linked through a 2 or 3 step-reaction sequence : the reactive alkyl halogen end of epichlorhydrin is first coupled to the hydroxyl groups of the dextran coating of the Fe_3O_4 crystals to give a terminal glycidyl ether derivative which can be used to link any amine containing molecule (peptide, protein, antibodies, ... or ammonia). The folate and the biotine are then bound to the aminated nanoparticle surface through the reaction between activated NHS ester and the amino groups. The NMRD profiles, which show the evolution of relaxivity (the increase of the relaxation rate of an aqueous suspension of particles containing 1millimole per liter of iron) with the magnetic field were recorded at 37°C on a Fast Field Cycling Relaxometer (Stelar, Mede, Italy). The relaxivities (r_1) were measured over a field range extending from 0.24 mT to 1.2 T. Additional measurements at 20, 60 and 300 MHz were respectively obtained on Minispec PC-20 and Mq 60 Series systems and on AMX 300 spectrometer (Bruker, Karlsruhe, Germany). The values of the average sizes and specific magnetizations were obtained from the fitting of the magnetometric curves (magnetometer VSM-NUVO, MOLSPIN, Newcastle Upon Tyne, UK).

RESULTS AND DISCUSSION:

Magnetometric curves of the diverse grafted nanoparticles are not significantly different (figure 1, table 1). The values of the diameter and of the specific magnetization given by the fitting of those curves by a Langevin function are similar for all particles. The vectorization has thus no marked influence on the magnetic and surface properties of the superparamagnetic crystal. The binding of the vector molecules to the superparamagnetic particles caused however an increase of the maximum of relaxivity of NMRD curves (figure 2, table 2). The theoretical fittings seems to indicate that the modification of the NMRD profiles results from a decrease of the water coefficient diffusion induced by the chemical modification. The vectorizing moiety thus apparently affects the properties of the coating. It has to be noted that because of the distribution of crystal size, the magnetometric size is, as expected, smaller than the relaxometric one. On the other hand, the magnetometric magnetization is higher than the relaxometric one.

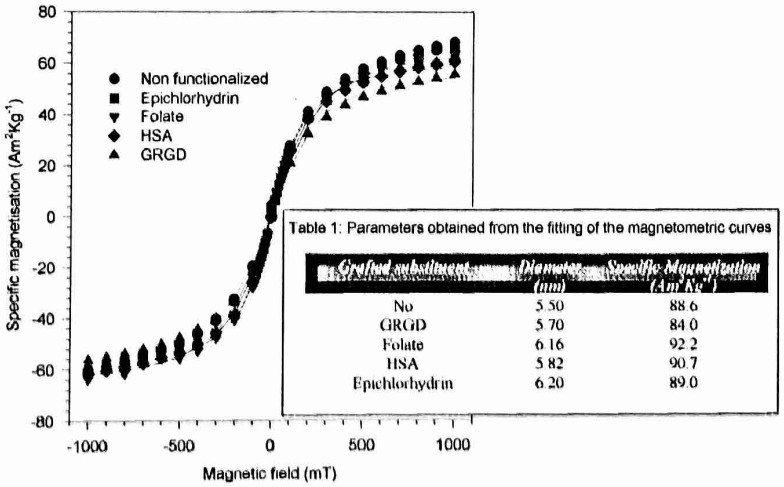

Table 1: Parameters obtained from the fitting of the magnetometric curves

Grafted substituent	Diameter (nm)	Specific Magnetization (Am^2Kg^{-1})
No	5.50	88.6
GRGD	5.70	84.0
Folate	6.16	92.2
HSA	5.82	90.7
Epichlorhydrin	6.20	89.0

Figure 1: Magnetometric curves of the original and grafted superparamagnetic particles

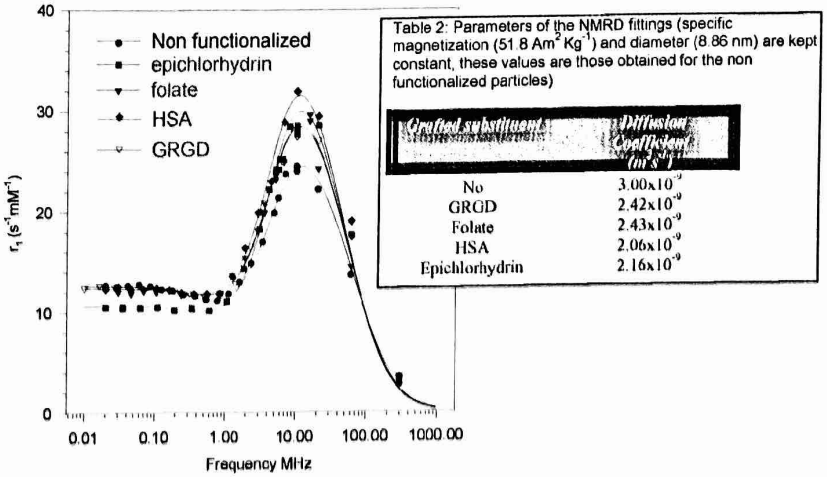

Table 2: Parameters of the NMRD fittings (specific magnetization (51.8 Am^2Kg^{-1}) and diameter (8.86 nm) are kept constant, these values are those obtained for the non functionalized particles)

Grafted substituent	Diffusion Coefficient (m^2s^{-1})
No	3.00×10^{-9}
GRGD	2.42×10^{-9}
Folate	2.43×10^{-9}
HSA	2.06×10^{-9}
Epichlorhydrin	2.16×10^{-9}

Figure 2: NMRD curves of the original and grafted superparamagnetic particles

CONCLUSIONS:

Functionalization of the dextran coating of nanomagnets does not affect significantly the magnetometric properties but causes an increase of the maximum amplitude of the NMRD curve. The increase of the relaxometric efficiency of the functionalized particles at the magnetic fields used in MRI should thus be beneficial for their use *in vivo*.

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