Microwave irradiation and Click chemistry for highly efficient and chemoselective multivalent iron oxide nanoparticle surface modification

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ABSTRACT

Magnetic nanoparticles (mNPs) are attractive candidates for magnetic immunoassays, cell labelling, imaging (MRI), and mediators of therapeutic hyperthermia as well as nanovectors for drug and gene delivery.[1] For all these applications mNPs will have to interact with other systems such as ligands, receptors, biomolecules, polymers. It is so crucial that mNPs surface have a define molecular structure. It must be surface functionalized with a precise control of quantities and orientation in order to meet the needs of specific applications.. In this contribution we will describe two methods for the functionalization of superparamagnetics γ-Fe_2O_3 nanoparticles coated with various molecules of the hydroxymethylene bisphosphonic acids (HMBP) family. Depending on HMBP functionalities we will describe improvement of classical peptide bond formation by microwave as well as the uses of click chemistry approaches for the functionalization of these mNPs

Keywords: magnetic nanoparticle, functionalization, microwave, click chemistry.

1 INTRODUCTION

Magnetic nanoparticles (mNPs) owing to their nanometric size and their magnetic properties are more and more used to improve efficiency and efficacy in various biomedical applications: targeted drug delivery, magnetic resonance imaging (MRI), immunoassays development, hyperthermia.[1-6] However, for this wide range of applications, most of the nanoparticles cannot be synthesized directly with their final properties. So mNPs surfaces must be tailored to improve biocompatibility properties and reduce aggregation but also to insert molecules on the particles surface with control of their architecture and surface density.[7] Several chemical methodologies are described for the mNPs functionalization.[8] The two more widespread being the classical technique of formation of amide, ester, carbamate, … bonds and the so called modern techniques of "click chemistry".[9-12] Both methodologies have their inconvenient and advantages but they still could be improved and new methodologies are to be found for a further development of nanotechnologies.

In our laboratory we developed iron oxide (γFe_2O_3) mNPs which are coated with molecules of the HMBP family.[13-16] These compounds are good chelators of metal ions by their bisphosphonic moiety and could possess other functionalities on their lateral chain which could be further modified to meet the needs of specific applications. For this work we used two compounds one already described, bearing an amino function on its side chain: Alendronate[17, 18] and a new HMBP bearing an alkyne function on its side chain (HMBP-yne).[19] We first evaluated the improvement of classical coupling by peptide bond formation using γFe_2O_3@Alendronate nanoplateform. We studied the coupling of a carboxylic dye: Rhodamine (Rh) and wished to exploit the potential of the microwave irradiation to reduce reaction time, and possibly improve the coupling processes. Then we adapted click chemistry techniques using γFe_2O_3@HMBP-yne nanoplateform. More specifically the CuAAC reaction [20-24] between alkyne function on mNPs and an azide functionalized carboxylic acid was performed.

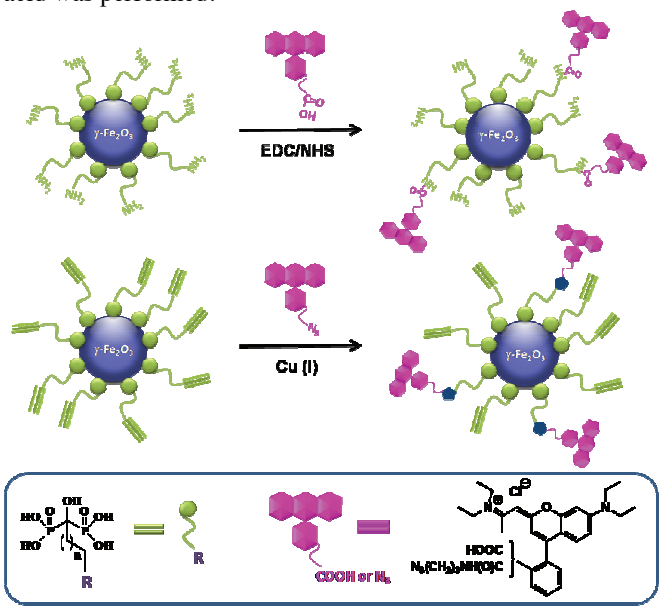

Fig.1: schematic principle of the studied mNPs functionalization

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2 MATERIALS AND METHODS

Alendronate was synthesized following literature protocol[25] and alkyne substituted hydroxymethylene bisphophonic acids (HMBP-yne) was obtained by adapting already described methodology.[26, 27] The synthesis of γFe_2O_3 nanocrystals and their surface coating with hydroxy methylene bisphosphonate molecules was done as previously described.[13-16] Free HMBPs were removed from coated particles thanks to a magnetic field and by centrifugation.

The peptide bond formation between Rhodamine (Rh) and γFe_2O_3@Alendronate was performed in water in a two step procedure (activation and conjugation) at room temperature or assisted by microwaves. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were used as coupling agent. Both activation and coupling reaction were done in classical conditions (i.e. respectively 2h and 24h reaction time at room temperature) or under microwave irradiation (i.e. respectively 5 min. and 2.5 min reaction time at power 50 W and maximum temperature = 42°C).

The click chemistry was performed by mixing for 48h, in water (pH=7), at room temperature, the γFe_2O_3@HMBP-yne with an azidomodified rrhodamine (10 eq.), sodium ascorbate (20%) and $CuSO_4$ (5%). For MW assisted reactions same conditions were used but with 10 min reaction time at 100°C. The modified mNPs were isolated with a magnet, and dialyzed (MCO = 50 000) to remove uncovalently bonded molecules.

UV-visible and IR spectra were respectively recorded on a Varian Cary 50 Scan UV-Visible spectrophotometer and on a Thermo Electron Corporation Nicolet 380 FTIR (KBr pellet) respectively. Fluorescence study was performed on a Perkin-Elmer LS50B Luminescence spectrofluorimeter. The size and the zeta potential of the nanocomplex were determined by dynamic laser light scattering (DLS) on a Nano-ZS (Red Badge) ZEN 3600 device (Malvern Instruments, Malvern, UK. The magnetic behaviour at room temperature of the as-synthesized nanoparticles is characterized using MIAplex® reader (Magnisense).

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3 RESULTS AND DISCUSSION

The amine groups present on the γFe_2O_3 @Alendronate surface were covalently conjugated with the carboxylic group of Rh *via* the EDC/NHS activation methods. Classical conditions and microwave assisted coupling were compared, moreover several stoechiometric ratios ($R = n_{Rh}/n_{\text{-}NH_2}$) of Rh versus amine groups ($\text{-}NH_2$) were evaluated. The coupling efficiency was investigated qualitatively using various measurement methods: infrared spectroscopy, dynamic light scattering, TEM, derivate magnetization (Miaplex®), and quantitatively using absorption and fluorescence spectroscopies. Figure 2 presents IR spectra comparison between γFe_2O_3@Alendronate and Rh coupled nanocrystals for R = 5, 50, using microwave treatment compared to starting nanoplateform and Rhodamine. Large changes, corresponding to Rh absorption, are observed all over the spectrum which tends to dominate when R increase suggesting an increase in the number of grafted Rh molecules. Moreover a new band appears around 1592 cm^{-1} arising from the formed peptide bond.

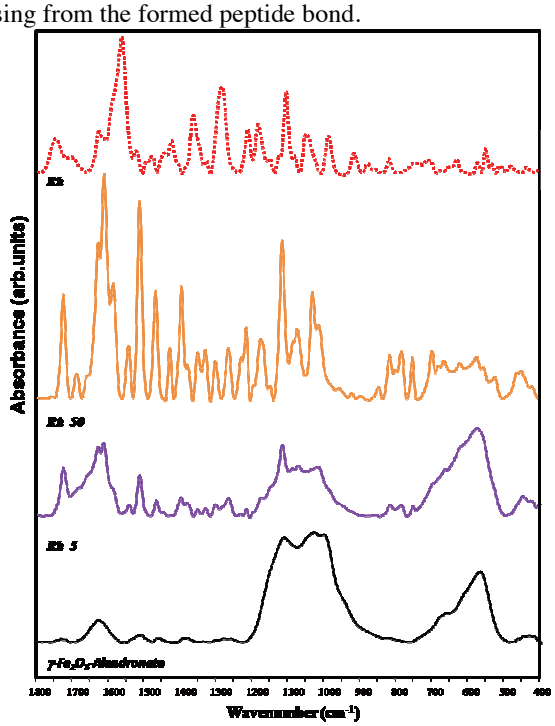

Fig.2: IR spectroscopy: γFe_2O_3@Alendronate compared to Rh microwave coupled nanocrystals performed at various R values. Insert: free Rhodamine B molecules.

This was correlated to an enhancement of the fluorescence emission (Fig. 3).

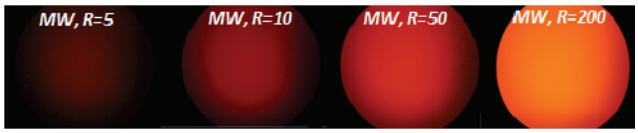

Fig.3: Photograph of fluorescent nanocrystal solution in water

Magnetic properties for the functionalized mNPs were kept almost unchanged. It also should be noted that when the amount of Rh on mNP is greatly increased stability of the nanocrystals is decreased.

For each experiment the number of Rh grafted at the surface of the mNP was evaluated using fluorescence spectroscopy and UV adsorption. Due to quenching phenomena of the mNP, measurements were done after removing the coating of the mNP by chemical treatment. The resulting supernatant containing Rh was then analyzed. The figure 4 represents the number of Rh molecules per γFe_2O_3@Alendronate nanoparticles. The grafting rate is gradually increased with the increase of R ratio. But for the

reactions done using the classical conditions a saturation corresponding to an average of 12 Rh per mNP was quickly reach whereas with microwave irradiation a number of 600 molecules per mNP could be obtained.

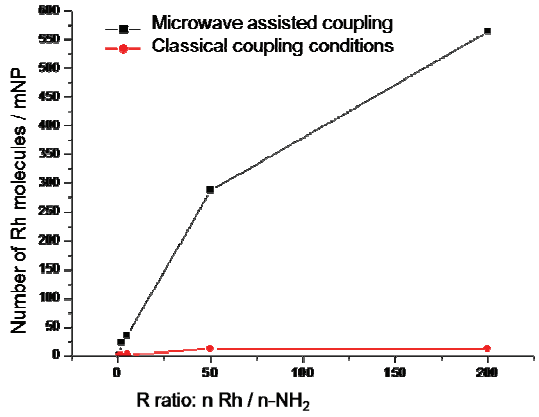

Fig.4: Variation of the number of Rh molecules grafted per mNP function of R ratio.

The second evaluation of mNP functionalization was done using click chemistry on γFe_2O_3@HMBP-yne. An azido rhodamine derivate was coupled using described conditions for CuAAC (Sharpless). An excess of ten equivalents was use in order to compare results with the previous approach. We also evaluated the input of microwave irradiation on the reaction. FTIR spectra were used to qualitatively compare the grafting efficiency (figure 5).

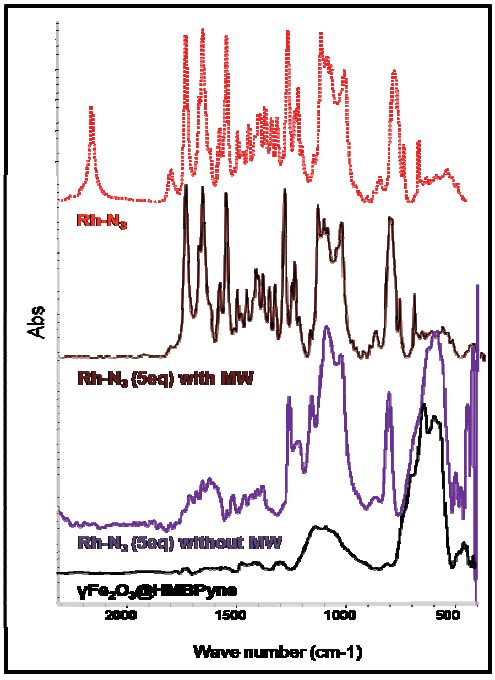

Fig.5: IR spectroscopy: clicked mNP compared to free clicked molecules.

Spectra showed that with or without MW the coupling occurs as vibration bands coming from Rhodamine are detectable. In the case of MW assisted reaction the Rhodamine adsorption completely dominate the spectra indicating an important increase in grafting. One must note that no vibration bands coming from azide function are present on both spectra of Rhodamine grafted mNPs in dicating that the coupling is covalent and that no unbounded azido rhodamine derivatives are remaining in the samples. Once again an enhancement of the fluorescence emission is observed when comparing reaction done with or without microwave (figure 6).

Fig.6: IR spectroscopy: clicked mNP compared to free clicked molecules.

After a chemical treatment to remove the coating of the mNP, analysis the supernatant by fluorescence spectroscopy was used to quantify the number of rhodamine per particle. For CuAAC reaction without the use of MW a number of approximatively 15 rhodamines/particle was found. In the same conditions using microwave activation the number of rhodamines/particle increased to 350 representing nearly a 25 fold increase. As for carbodiimide approach microwave treatment so represent a real improvement in grafting efficiency. Nevertheless, it could be note when comparing both carbodiimide and CuAAC approaches using the same excess in Rhodamine derivatives (10 eq) CuACC without MW is nearly as efficient as MW assisted carbodiimide coupling. Moreover similar high grafting (around 300 molecules per particle) are obtained under MW irradiation with both methodology but using 5 times less reactant with MW-CuAAC (10eq.) than with MW carbodiimide (>50 eq.). Both results indicated the higher efficiency of CuACC chemistry for the mNPs functionalization.

4 CONCLUSION

We successfully evaluated two different methodologies for the functionalization of mNPs. The classical methodology using coupling by peptide bond formation was greatly improved by the use of microwave technology. Whatever the method used to deduce the grafting yield it can easily be seen that microwave coupling is far more effective. The grafting rate is gradually increased with the increase of Rh stoichiometry. In contrast to microwave treatment, increasing the initial *R* ratio does not induce an increase in the average number of molecules per nanocrystal and a saturation corresponding to an average of 12 Rhodamine molecules per nanocrystal is quickly obtained. Such an amount of fluorophore per nanoparticle is currently found

[28, 29] in the literature for such high molecular weight aromatic molecules. Hence, using the microwave we manage to have approximately a 50 fold increase in the number of molecules per nanoparticle. Moreover we show that the amount of Rhodamine on the surface could be tuned using various stoichiometric ratios. We also evaluated CuAAC reaction to graft the same fluorophore onto the mNPs surface. We demonstrate that this methodology is more efficient and could also be greatly enhanced by using microwave activation. So with CuAAC this time by varying the methodology (with or without MW) we could tailored the number of molecules onto the surface. Moreover using CuAAC, without MW, we could grafted small but sufficient amount of fluorophore onto the nanoparticle with still remaining a lot of alkynes functionalities on the surface. So this already bimodal nanoplateform (superparamagnetic and fluorescent properties) could be again derivatized with other azide functionality or even with another chemoselective reaction such as thiol-yne chemistry[30] thus allowing multivalent functionality.

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