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Selective functionalization of mesoporous silica nanoparticles with ibuprofen and Gd(III) chelates: a new probe for potential theranostic applications.

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Organo-modified mesoporous silica nanoparticles, loaded with ibuprofen into the pores and functionalized on the external surface with a stable Gd(III)-DOTA-monoamide chelate, were prepared and explored as potential theranostic probes.

A theranostic device is a multifunctional nanoparticle (NP) that combines drug molecules and diagnostic agents in the same platform and it can be simultaneously exploited for diagnosis and therapy.1 In the last decade, great efforts have been made in this field, as testified by more than 400 articles published in the literature.2 Various nanosystems have been proposed for the construction of novel theranostic agents, including inorganic NPs (i.e. silica, gold), polymeric micelles and liposomes, often embedded in the gel synthesis by using aminopropyltriethoxysilane. NPs with spherical morphology and size of 90±30 nm (as indicated by TEM micrographs, Fig. 1A, C) were obtained. Micrographs collected at high magnifications (Fig. 1A) indicate the presence of a pore array, high chemical versatility.

The first to publish their findings on these drug loaded MSNs was Vallet-Regí et al. in 2001.5 More recently, MSNs carrying a number of different drugs (anti-inflammatory and anti-cancer molecules) and decorated with biocompatible agents (i.e. phospholipids layers)4 and target molecules (folic acid, peptides,...)7 have been reported.

In parallel, MSNs functionalized with Gd(III) chelates have been studied as potential T<sub>1</sub> MRI contrast agents. The efficacy of a MRI probe is measured in terms of the relaxivity parameter, $r_1$ [mM<sup>-1</sup> s<sup>-1</sup>], which indicates the variation in the relaxation rates of water protons normalized to 1 mM Gd<sup>3+</sup> ion concentration. Initial studies on the use of Gd(III)-based chelates with MSN include different shapes and sizes of the NPs to improve the $r_1$ relaxivity of the system for cell tracking and angiographic MRI applications.8 In this field we have optimized the relaxometric properties of Gd(III) chelates conjugated to mesoporous silica, obtaining high $r_1$ values per Gd<sup>3+</sup> (79 mM<sup>-1</sup> s<sup>-1</sup>) and per particle (≈ 65800 mM<sup>-1</sup> s<sup>-1</sup>) at 20 MHz and 310 K. These remarkable values have been obtained by a careful design of the system aiming to reduce the interactions between the silica surface and the Gd(III) chelates and improve the water accessibility to the metal probe.9-11

Recently, the combination of silica particles with drug molecules (e.g. doxorubicin) and paramagnetic metal oxides (Fe<sub>3</sub>O<sub>4</sub>), typically working as negative contrast agents (T<sub>2</sub>), have been greatly investigated in the literature.12 On the contrary, theranostic systems functionalized with T<sub>1</sub>-MRI Gd(III)-based contrast agents, generally used to improve the quality and diagnostic content of the MRI images in drug delivery and therapy applications, appears only marginally explored. In this contest, M.-A. Fortin et al. have very recently published a functional Gd-based theranostic agent grafting GdDTPA-like chelates and PEG on the external surface of MCM-48 NPs and loading daunorubicin into the pores.13

In this work, an organo-modified hybrid MSN consisting of NPs of 90±30 nm size and functionalized with -NH<sub>2</sub> groups, was selected as platform. A neutral GdDOTA-monoamide derivative (GdDOTAMA, Fig. 1) was selected as potential MRI probe. This macroyclic complex, bearing a pendant arm with a terminal carboxylic functional group that can be used for conjugation to the silica surface, is characterized by high thermodynamic stability and kinetic inertness.14,15 Ibuprofen was used as a typical drug model. The different silica domains were selectively exploited to link GdDOTAMA on the external surface of NPs and to accommodate ibuprofen into the channels.

MSNs were prepared through a sol–gel procedure reported in the literature.11 The amino functional groups were introduced during the gel synthesis by using aminopropyltriethoxysilane. NPs with spherical morphology and size of 90±30 nm (as indicated by TEM micrographs, Fig. 1A, C) were obtained. Micrographs collected at high magnifications (Fig. 1A) indicate the presence of a pore array,
The ibuprofen uptake was quantified by measuring the amount of free drug in the washing medium hexane with UV-Visible spectroscopy at 273 nm (see Fig. S1). The ibuprofen concentration absorbed by MSN is 0.60 (±0.02) mmol/g (12.4 wt%). A physico-chemical analysis of the hybrid material was carried out before Gd-chelate functionalization to determine the effective drug impregnation. Thus, the mesopores filling with ibuprofen molecules was evaluated by N2 adsorption. The ibuprofen inclusion produces a significant change of the pore size distribution of MSN. The two pores populations at 22 and 37 Å of free MSNs decrease in intensity after drug impregnation. A new peak at ca. 14 Å (Fig. 1D) and a decrease of the pores volume from 1.52 cm³/g to 0.87 cm³/g were observed. These results confirm that the ibuprofen molecules are located inside the pores.

Further confirmations come from infrared spectroscopy analysis. IR spectrum of the functionalized material shows the peaks typical of the organo-modified silica (i.e. bands at 3350 and 3290 cm⁻¹ and at 1595 cm⁻¹ (Fig. S2), assigned to the asymmetric and symmetric stretching and bending modes of the -NH2 species, respectively) along with a sharp band at 1710 cm⁻¹, attributed to the stretching mode of the COOH group of ibuprofen. Two peaks at 1510 and 1390 cm⁻¹, typical of C=C stretching of the aromatic ring and the bending mode of CH3 groups of drug, respectively, are also detected (Fig. 2, right and Fig. S3).

The percentage of ibuprofen released from MSNs over time was calculated by using UV-Visible spectroscopy (Figure 2). Prior to the analysis, the sample was pelleted and immersed into SBF solution (simulated body fluid), at neutral pH and 298 K. These experimental conditions promote the deprotonation of the COOH group of ibuprofen and the conversion of the NH2 functionalities of MSN to NH3⁺ (see IR data reported in Fig. S4).

The ibuprofen released during the first 8 h followed a linear behaviour with zero order kinetics (Fig. S5), typical of systems in which the drug strongly interacts (through ionic bond) with the silica surface. After the first day, the release further decreases with time and reaches a maximum value of 57% before levelling off.

After conjugation of the GdDOTAMA chelates to the surface, the final concentration of Gd³⁺ present in the material was determined by inductively coupled plasma optical emission spectrometry (ICP-OES). 0.01 mmol of Gd per gram of material was found, corresponding to approximately 1290 (±50) complexes per particle. Only a small fraction of NH2 groups, mainly located on the external surface, were involved in the coupling procedure (ca. 0.3% of the total amount, estimated to be 3 mmol g⁻¹, by thermogravimetric analysis (Fig. S6)). The IR spectrum of the final material shows a main absorption at 1640 cm⁻¹ attributed to the bending mode of the amide group (Fig. S7). The presence of this signal is a clear evidence that the Gd(III) chelates are bound to the organo-silica surface. In addition, it is important to note that the intensity of ibuprofen IR absorptions appears ca. 90% preserved after the coupling reaction (Fig. S7).
suspension (20 MHz, 310 K, pH = 7) increases to 23.7 mM which exhibited nuclear magnetic relaxation efficacy of the present system appears is known about macromolecular paramagnetic complexes, a strong 1300 Gd(III) chelate anchored to the surface of a NP. Based on what Magnetic Relaxation Dispersion (NMRD) profiles (Fig. 3). frequency range 1-70 MHz, obtaining the so-called Nuclear measured at 310 K as a function of the magnetic field over the materials is very similar and quite typical of slowly tumbling systems. Hence, low field data, those most affected by electronic relaxation, were not included in data analysis, in accordance with a well-established approach. \( \Delta^2 \) has a value of (7.7±0.6)×10^{-18} s^{-2} and \( \tau_v \) of (16±1) ps, quite similar to those found for strictly related systems.11

To strengthen our understanding on the relaxometric properties of the material before and after the release of ibuprofen, \( r_1 \) was measured at 310 K as a function of the magnetic field over the frequency range 1-70 MHz, obtaining the so-called Nuclear Magnetic Relaxation Dispersion (NMRD) profiles (Fig. 3).19 The nature of the magnetic field dependence of relaxivity for both materials is very similar and quite typical of slowly tumbling systems, characterized by a broad peak with a maximum around 20 MHz. However, the amplitudes of the two NMRD profiles are clearly different. We can observe a distinct increase in relaxivity over the entire frequency range investigated following the partial release of the drug. The contributions of the different physicochemical influences on \( r_1 \) can be evaluated quantitatively by analyzing the NMRD profiles according to well-established theoretical models. For molecular gadolinium agents the shape and amplitude of the frequency dependence of \( r_1 \) are well described by the equations of Solomon-Bloembergen-Morgan.19 The key parameters that determine the relaxivity observed are: the number \( q \) of metal-bound water molecules, the correlation time for tumbling of the agent \( (\tau_a) \), the mean lifetime of the water molecule in the coordination sphere of the paramagnetic ion \( (\tau_{bw}) \), the parameters characterizing the electron spin relaxation, i.e. the electronic correlation time for the modulation of the zero-field-splitting interaction \( (\tau_c) \) and the mean square zero-field-splitting energy \( (\Delta^2) \). In the case of metal chelates conjugated to scaffolds of nanometric size, the rotational dynamics is more appropriately evaluated in terms of the Lipari–Szabo model-free approach that accounts for the occurrence of a rapid local rotation of the complex \( (\tau_{RL}) \) superimposed to the global motion of the nanoparticle \( (\tau_{G}) \).20 This description is particularly appropriate for our GdDOTAMA functionalized MSNs due to the presence of a flexible linker connecting the Gd complexes to the MSN surface. The coupling of local and global motions is described by the order parameter \( S^2 \) whose value lies between 1 (global and local motions coincide) and 0 (local and global motion are completely independent).

Using known values for \( q (q = 1) \) and for the distance \( \frac{R_{GdHw}}{V_{GdHw}} \) between the metal ion and the protons of the coordinated water molecule \( (\frac{R_{GdHw}}{V_{GdHw}} = 3.0 \text{ Å}) \) and fixing \( \tau_{bw} \) to 0.1 ms, in analogy with previous studies,10,11 we have analyzed the experimental data in terms of five adjustable parameters: \( \Delta^2 \), \( \tau_a \), \( \tau_{RL} \), \( \tau_M \), and \( S^2 \) as (Table 1). The parameters for electronic relaxation were used as empirical fitting parameters and have no real physical meaning for macromolecular systems. The best-fit curves were calculated using the parameters of Table 1. B) \( r_1 \) values for GdDOTAMA functionalized MSNs before (Gd-1) and after partial drug release (Gd-2) at 20, 40 and 60 MHz.

The increase of \( r_1 \) for the sample after partial drug release is associated with the variation of two dynamic parameters that characterize the system: the local motion of the complex around the linker and the exchange rate of the coordinated water molecule. This change in \( r_1 \) does not result from a variation of the \( q \) number during the drug release because GdDOTA-monoamide chelates are typically very stable and the coordinated water
molecule is not affected by the presence of ibuprofen in close proximity. Indeed, the relaxation rate value (measured at 20 MHz and 298 K) of pure GdDOTAMA solution, treated with increased amount of ibuprofen sodium salt, remained constant (Fig. S9). Specifically, the release of the drug from the silica is accompanied by an acceleration of the water exchange ($r_1$ decreases by ca. 45%) and by a faster local rotation of the complex ($r_2$ decreases by ca. 25%). For the sample with ibuprofen impregnated into the pores, $r_1M$ assumes the value of 1.3 $\mu$s (310 K), which is sensibly longer than those found for related GdDOTA-monoamide complexes. Then, the combination of a long $r_1M$ with a restricted local motion of the complex ($r_2L = 23$ ns) is indicative of an occurrence of an interaction of the Gd(III) complex with the surface of MSN.

Clearly, the value of $r_1M$ plays a dominant role and its decrease largely offsets the negative effect associated with the less restricted local rotation of the complex. Furthermore, the $r_1$ enhancement and the variation of the dynamic parameters of the Gd-chelates strongly suggest the presence of interactions between the complexes and the drug molecules. In particular, these interactions must involve complexes located on the edge or in close proximity of the entrance of the pores. It is fairly obvious to assume that interactions of electrostatic nature, also involving a number of H-bonded water molecules, make the exchange of the coordinated water with the bulk more difficult and limit the rotational degree of freedom of the complex around the linker. The partial release of the drug, especially the molecules nearest the entrance of the pores, removes this type of interactions allowing easier access of water to the metal site and increasing the rotational flexibility of the chelates. Previous studies have shown that the local ionic environment of the complex influences the process of coordinated water exchange (both prototropic and of the whole molecule) and then $r_1$. Then, the effect is particularly relevant in the case of systems in slow exchange ($r_1M > ca.$ 5 $\mu$s) but it is likely that also in this case a similar mechanism might contribute to the variations in $r_1$ observed. In particular, the release of the drug changes the ionic environment and thus the nature of the second sphere of hydration of the Gd(III) centers through the replacement of the carboxylate groups with other anions, to a large extent chlorides. In turn, this influences the value of $r_2$ and hence relaxivity.

### Notes and references


### Conclusions

Multifunctional mesoporous silica nanoparticles, selectively functionalized on the external surface with thermodynamically stable and kinetically inert Gd(III) chelates and loaded inside the pores with ibuprofen molecules, were successfully obtained and characterized. The experimental results suggest that mutual interactions between ibuprofen, Gd-chelates and the silica surface determine the effectiveness of the final theranostic system. Both the relaxivity of the Gd-based MSNs and the kinetics of the drug release are markedly influenced by the presence of one or the other component of the final material. Therefore, the release of ibuprofen is slowed down by anchoring the Gd-chelates on the surface of the NPs and the relaxivity increases significantly following the partial release of the drug. The ability to modulate the functional properties of the system, through controllable chemical procedures, is of great interest for the development of intelligent theranostic agents. For example, it might be possible to follow the process of drug release by monitoring the resulting changes in the relaxation rate. For a development in this direction, an optimization of all the components of the theranostic probe is required. New formulations based on Gd(III) chelates with improved efficacy, the modulation of the interaction MRI probes-drug molecules through a suitable choice of the length and chemical nature of the linker and of the electrical charge of the metal complex, the modification of the surface properties of NPs through the chemical transformation of the exposed groups are being studied and will be the subject of future work.

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