Journal Name

COMMUNICATION

A Structural and ^{1}H NMR Relaxometric Study on Novel Layered Carboxyalkylaminophosphonate Nanocrystals with Gd(III) Ions Located into the Framework.

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Novel Gd(III) carboxyalkylphosphonate nanocrystals were synthesized under mild hydrothermal conditions. Structural properties and ^{1}H NMR relaxometric behaviour in aqueous solution as a function of the magnetic field strength were investigated, aiming to evaluate the chemical local environment of the paramagnetic centres and their interaction and affinity with water molecules.

In the last decade, several inorganic and hybrid organic/inorganic nanoparticles (NPs) functionalized with paramagnetic ions (both transition metals and f elements)[1-3] or with metal chelates[4-6] have been designed and employed for a number of applications, especially in diagnostics and bioimaging. Zeolites activated with lanthanide ions (Gd(III) and Eu(III)) have been considered for magnetic resonance, optical and/or multimodal imaging applications.[7] Mesoporous silica nanoparticles containing Gd(III) ions into the inorganic framework[8] or functionalized on the external surface with highly stable Gd(III)-chelates[9-12] have been thoroughly investigated. LnF$_3$/NaLnF$_4$ and Ln$_2$O$_3$ NPs are being actively studied and used in a number of biomedical applications.[13] Recently, detailed studies were carried out to elucidate the mechanisms underlying their interaction with water molecules and the role of the surface in determining their relaxometric properties.[14-15] More recently, Gd(III)-based metal organic frameworks (MOFs) were also examined by relaxometric techniques and proposed as potential T_1 and T_2 MRI contrast agents.[16-17]

However, despite the many examples reported in the literature, the direct synthesis of nanosized materials containing Gd(III), using low cost and facile one-pot procedures, remains an ambitious yet not fulfilled goal. Lanthanide phosphonates have been extensively studied during the last few years owing to their facile synthesis and

high structural versatility.[18] Phosphonic groups display a good affinity towards Ln(III) ions giving rise to highly insoluble compounds. In addition, the proper choice of the molecular geometry of the building block could allow the presence of free functional groups, which serve as anchoring groups or for bio-conjugation.

In light of these considerations, a new family of layered carboxyalkyl-phosphonate NPs containing paramagnetic Gd^{3+} ions as framework metal have been prepared and characterized in this work. On this regard, phosphonates/phosphinates are known to be stable compounds and they have been largely employed as stable chelators. As an example, Gd phosphate based nanoparticles have been recently employed as contrast agent (CA) in MR imaging both self-standing and conjugated with DNA single strands.[19] Among phosphonates, amino-bis(methylenephosphonates) possess an affinity towards lanthanide ions comparable to other ligands.[20] In a recent paper, carboxyalkylamino-N,N-bis(methylphosphonates) were used as ligands by some of us for the synthesis of layered Ln(III) derivatives (with Ln = Eu, Tb, Er, Yb).[21] The synthetic conditions and the high insolubility of these compounds allowed to obtain stable aqueous dispersion of elongated Ln(III) phosphonate nanoparticles with size ranging from 200 to 500 nm.

In this communication we report on the synthesis and characterization of three novel Gd(III) carboxyphosphonates, obtained by reaction of Gd(NO$_3$)$_3$ with N-phosphonomethylglycine (hereafter H$_3$L^1), 4-(bis(phosphonomethyl)amino)butanoic (hereafter H$_5$L^2) and 6-(bis(phosphonomethyl)amino)caproic (hereafter H$_5$L^3) acids under mild hydrothermal conditions. The molecular structures of the three carboxyalkylaminophosphonic acids are shown in the Scheme 1.

A comparison of the structural and ^{1}H NMR relaxometric behavior in aqueous solution of all of the samples was undertaken, aiming to

evaluate the chemical environment of the paramagnetic ions and their interaction/affinity with water molecules.

The reaction of $Gd(NO_3)_3$ with the three ligands in water solution led to the formation of layered Gd(III) phosphonate materials with formula $Gd(HL^1)_2$,(**1**), $Gd(H_2O)(H_2L^2)$, (**2**) and $Gd(H_2O)(H_2L^3)$ (**3**). The structures of two of these phosphonates were solved *ab-initio* from powder X-ray diffraction (PXRD) data and fully characterised by Fourier transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA) and transmission electron microscopy (TEM).
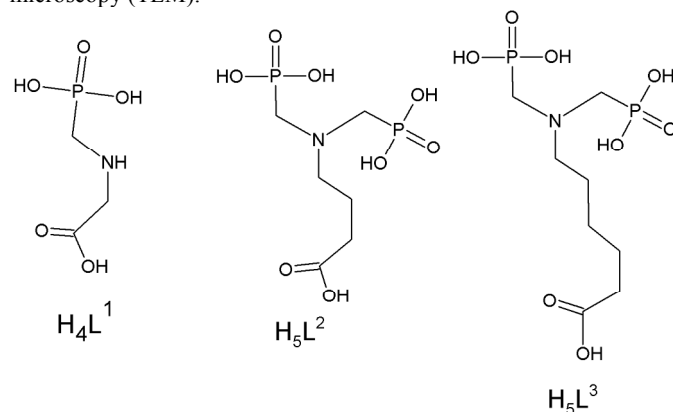
Scheme 1. Molecular structure of the three polidentate phosphonate ligands.

Compound **1** has a layered structure, in which the layers are composed of PO_3C tetrahedra and GdO_7 coordination polyhedra. The amino-carboxylic chains point towards the interlayer space; the layer was formed by the interconnection between the Gd(III) ions (showed as violet atoms) and phosphonate ligands: one gadolinium ion joins six oxygen atoms coming from five different phosphonate tetrahedra and one carboxylic oxygen belonging to the adjacent glycine chain. The coordination of the COO^- groups to the Gd(III) was also proved by recording the FT-IR spectrum of sample **1** that shows a band at 1610 cm^{-1} (asymmetric stretching of the COO^-) that falls at lower frequencies if compared to typical absorption of free carboxylate groups (Fig. 15S.) For comparison the FT-IR spectrum of pure H_4L^1 is also reported in figure 16S. The asymmetric stretching of carboxylate falls at 1732 cm^{-1}, which is typical of uncoordinated carboxylic groups.The organic moieties are pending from the inorganic framework, occupying the region among the chains. In this compound, the Gd(III) coordination sphere does not contain water molecules, as also confirmed by TGA analysis. Furthermore, there is a network of H-bonds involving the carboxylate groups and the free P-O group. One of the carboxylic oxygen atoms (O15) strongly interacts, via H-bond, with the oxygen atom of the phosphonic group belonging to the adjacent layer (see Figure 1S). Another O atom of the doubly connected phosphonic group points towards the N- atom of the amino group of the adjacent glycine moiety (P18-O20···N3 = 2.85 Å), suggesting that a non covalent interaction exists between these two atoms, or better, that the nitrogen is protonated by the neighboring PO-H group (Figure 2S).

The structure of compound **2** was not solved because the crystallinity of the compound was too low, and the indexing process was unsuccessful. However, the diffraction pattern of this phase was similar to that of compound **3** with a strong basal peak at low 2θ angle, which can be ascribed to the layer stacking (Figure 3S). The d-spacing is about 14.4 Å that is compatible with an interdigitated arrangement of the butanoic chains, in agreement with what observed for compound **3** (containing hexanoic chains) with a basal distance of about 18 Å.

Compound **3** shows a layered structure with layers composed either of GdO_9 distorted tetragonal antiprism and PO_3C tetrahedra, belonging to the diphosphonic moieties. The Gd ion is directly bound to a water molecule. The inorganic layers are constituted of a corrugated plane of nine-coordinate Gd ions with a distorted tetragonal antiprism coordination. The average Gd-O distances were in the range 2.2-2.8 Å with two long bond distances of about 2.6 to 2.8 Å (Gd-O2 = 2.8 Å and Gd-O4 = 2.6 Å). The Gd-Ow distance (Ow = water molecule) is about 2.25 Å. These polyedra are connected through the oxygen atoms of the two phosphonic moieties, above and below the plane of the layer. One PO_3C tetrahedron is internal to the sheet (P8) and it is connected to four different Gd(III) ions. The other phosphonate tetrahedron (P7) is connected only to one Gd(III). The carboxyalkyl chains are placed into the interlayer region in an interdigitated disposition. The chains are in bent conformation because one oxygen atom of the carboxylic group (O19) is involved in a H-bond with the free oxygen atom of the phosphonic group (O5) belonging to the inorganic layer, with distance P8-O5···O19 = 2.5 Å (Figure 4S).

The structures of compounds **1** and **3** are shown in Figure 1.
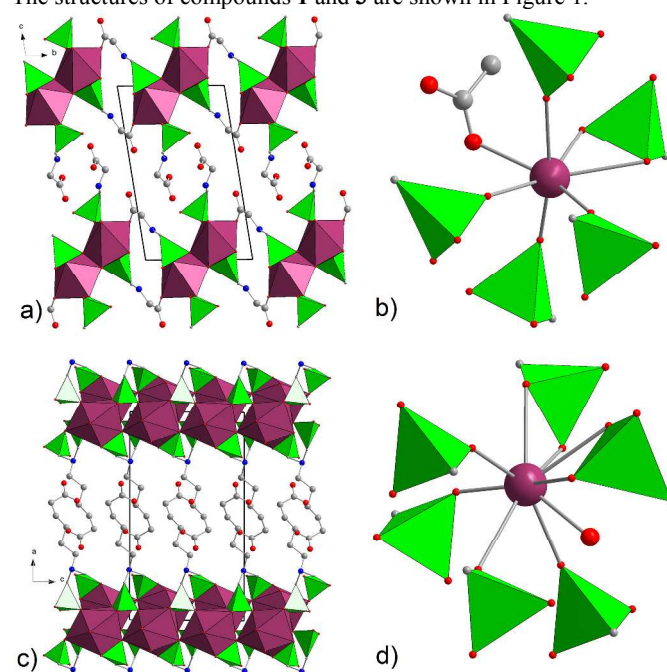

Figure 1. Polyhedral representation of the structure and coordination environment of Gd of **1** (a and b, respectively) and **3** (c and d, respectively). Gd polyhedra are represented in light violet, PO_3C tetrahedra are represented in green.

Thermogravimetric curves are shown in Figure 5S. Compound **1** possess high thermal stability (up to 300 °C) and no structural water molecules are present, in agreement with the X-ray structure. Compound **2** shows a neat weight loss of about 2% below 200 °C that is compatible with one Gd coordinated water molecule. Compound **3** also shows a neat weight loss of about 3% at 150 °C corresponding to the evaporation of one Gd-coordinated water molecule. In agreement to the crystal structure, no intercalated water is present.

Stable dispersions of compounds **1-3** have been obtained at neutral pH by slowly adding NaOH 0.1 M that favours the dispersion of the particles by acid-base interactions and then the pH was adjusted to 7 with HCl. The dispersions are stable over several days as shown in Figure 9S. The samples, before and after sonication in water suspension, were examined by field emission scanning (FE-SEM)

and transmission electron microscopy (TEM), respectively. The micrographs are reported in Figure 2. The crystal morphology of the three samples reflects their layered structure, as they are flat elongated platelet crystals with prismatic shape. In the FE-SEM images (Figures 2a, c, and e), the nanocrystals are close to each other, forming aggregates of few microns. After sonication, the nanocrystals appeared better separated and they have average size of about 100 to 200 nm measured along the short axis and up to 600-700 nm measured along the elongation axis (Figures 2b, d, and f).
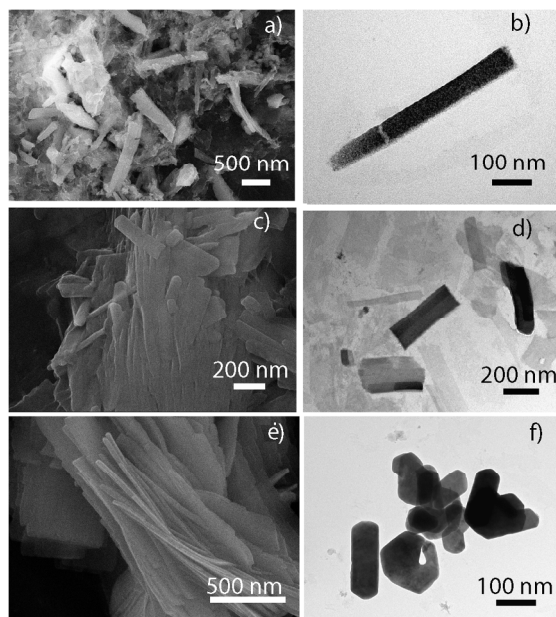

Figure 2. FE-SEM (left) and TEM (right) images of **1** (a, b); **2** (c,d); **3** (e,f) nanocrystals.

The ^{1}H NMR relaxometric behaviour of the three compounds in aqueous suspension was investigated at 298 K and neutral pH. The longitudinal (r_1) and transverse (r_2) proton relaxivities represent key parameters that describe the efficacy of the paramagnetic ion in increasing the nuclear magnetic relaxation rates of the water protons, normalised to a concentration of 1 mM. The r_1 values at 0.47 and 1.41 T are reported in Table 1. The r_2/r_1 values at 0.47 T (Table 1) are low (especially for sample **1** and **2**), thus indicating that such materials behave as positive MRI contrast agents.

Table 1. r_1 values (per Gd) measured at 0.47 and 1.41 T (298 K).

	r_1 (mM^{-1}s^{-1}) 0.47 T	r_1 (mM^{-1}s^{-1}) 1.41 T	r_2/r_1 0.47 T
1	3.9	4.7	1.2
2	1.4	1.7	1.7
3	0.6	0.6	2.8

The r_1 enhancement stems from the dipolar coupling between the proton nuclei of the water molecules and the metal ions, which involves two basic mechanisms: a short-range interaction with either metal-bound water molecules (inner sphere contribution, IS) or H-bonded to polar groups of the ligand (SS contribution) and a long-range interaction with water molecules of the bulk diffusing next to the surface (outer sphere contribution, OS). The magnetic coupling

with the water protons in many inorganic NPs has a marked dependence on the position of the Gd(III) ions within the particle. The ions located in the core of the particle may contribute to r_1 mainly through the OS mechanism, which gradually becomes less important with the increase of their distance from the surface. The ions on the surface, instead, contribute also through the SS mechanism and, in the case of coordinated waters, also through the IS mechanism. Therefore, the calculated value of the ionic relaxivity (per Gd) represents only an average value between limit situations characterized by quite different contributions. However, a similar result may occur when the water molecules have free access to the inside of the sample, but their rate of diffusion, and hence exchange with the bulk, is slow.

The ^{1}H relaxivity as a function of the proton Larmor frequency (Nuclear Magnetic Resonance Dispersion, NMRD)[22] was measured at 298 K over the range 0.01-70 MHz. The NMRD profile of **3** is characterized by low r_1 values over the entire frequency range, to indicate that only the OS mechanism is operative. As already noted above, the low r_1 values reflect an average between ions closer to the surface whose interaction with the solvent is relatively strong and ions embedded in the nanoparticle that do not contribute at all to the observed relaxivity. A fit of the data to the OS relaxation theory provides an effective distance of closest approach of 9.7 Å for the outer-sphere water molecules with a standard value for the diffusion coefficient of 2.3×10^{-5} cm^2 s^{-1} at 298 K.[23]

On the other hand, the NMRD profiles of **1** and **2** are characterized by a low frequency plateau (0.01-2 MHz) and a broad peak centred about 50 MHz (Fig. 3). This shape is typically found in slowly tumbling systems endowed with a rotational correlation time τ_R of the order of a few hundreds of ps. The dependence of the profile on τ_R implies that, in addition to the OS mechanism, also the SS relaxation mechanisms contribute to r_1. A contribution from IS water molecules can be excluded based on the relatively low r_1 values. In fact, a good fit could be obtained by considering the presence of SS water molecules per Gd ($^{SS}q = 1$ for **1** and $^{SS}q = 0.3$ for **2**) at an average distance, ^{SS}r, of 3.5 Å and with a $^{SS}\tau_R$ value of 275 and 240 ps for **1** and **2**, respectively (Fig. 3) (additional details are reported in Table S1).
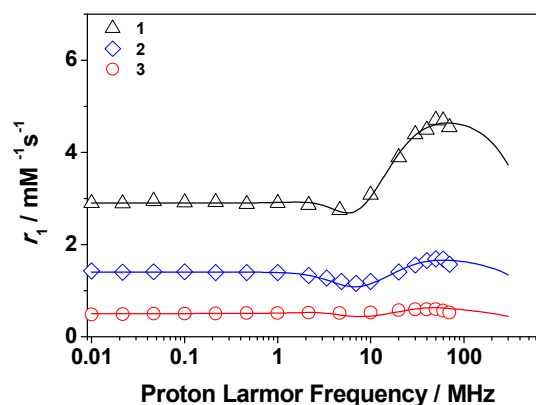

Figure 3. $1/T_1$ NMRD profiles for **1** ($\triangle$), **2** ($\diamond$) and **3** ($\circ$) at 298 K.

Conclusions

The paper reports on a comparative study on the structure and ^{1}H-NMR relaxometric properties of three novel Gd-carboxyalkyl phosphonates with nanometric crystal size and with different length of the alkyl chains. The compounds are characterized by layered structure containing Gd(III) ions located into the layers with different coordination spheres. The relaxation properties show a

direct dependence on the length of the alkyl chains and therefore on the hydrophilic/hydrophobic character of the chemical environment of the paramagnetic site that determines its interaction with water. In fact, compound **1**, with glycine moiety and thus higher hydrophilicity, displays the best relaxometric performances, owing to its strongest SS contribution, in good agreement with the crystal structure. It is worth noting that the relaxivity of **1** (per Gd) is quite similar to that of the clinically used MRI probes.

A proper selection of the precursors used in the Ln-carboxyalkylaminophosphonates is essential as it allows modulating the physico-chemical and relaxometric properties of the final nanoparticles.

Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [Instrumental details, synthetic procedures, supplementary TEM and structural figures, thermogravimetric data, complete crystallographic data for **1** and **3**]. See DOI: 10.1039/c000000x/

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