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Confinement of a tris-aqua Gd(III) complex in silica nanoparticles leads to high stability, high relaxivity and supresses anion binding.

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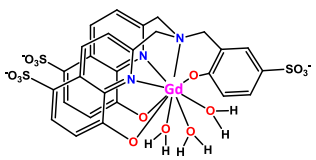
The water soluble tris-aqua complex $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ based on a hexadentate hydroxyquinoline ligand shows high thermodynamic stability, and high relaxivity (12.54 $mM^{-1}s^{-1}$ at 1.2 T). Its non-covalent confinement in 25 nm silica nanoparticles prevents transmetallation and endogenous anion binding and leads to higher relaxivity over a wide range of magnetic fields.

Mono-aqua gadolinium complexes are widely used in magnetic resonance imaging (MRI) clinical examinations to increase the image contrast.[1] Due to their higher spatial resolution and sensitivity, high (3 T) and ultrahigh (> 3 T) MRI fields are increasingly used in clinical and in preclinical applications, respectively.[2 3] However, the efficacy of the commercial contrast agents (measured by their relaxivity (r_1) defined as the induced increase of the water proton relaxation rate $R_1 = 1/T_1$ per mM of dissolved contrast agent) is ill suited for field values ≥ 3 T. A straightforward way to improve the efficacy of Gd(III) complexes at high field consists in increasing the number (q) of water molecules coordinated to the metal center by reducing the denticity of the Gd(III)-binding chelate. However, this usually reduces the thermodynamic and kinetic stability compared to mono-aqua complexes so that the toxic Gd(III) ion is released in biological environment.[1] Moreover, in bis- or tris-aqua complexes, the Gd(III) bound water molecules are often displaced by endogenous anions such as phosphate or carbonate leading to a lower relaxivity. As a result, studies on bis-aqua and tris-aqua Gd(III) complexes remain limited[4-12] compared to the very large number of scientific publications reporting on mono-aqua complexes. Scarce attention has also been given to the incorporation of multi-aqua Gd(III) complexes into nanocarriers such as silica nanoparticles,[13, 14] although this is an attractive way to deliver a large number of Gd(III) complexes to a single desired site and to increase the efficacy of each Gd reporter.[13, 15-21] Silica nanoparticles are particularly attractive carriers because they are not toxic and their surface can be easily functionalized for targeting biomolecules.[22]

We recently reported that the q = 0 - 1 Gd(III) complexes of ligands containing hydrophobic groups such as pyridine or hydroxyquinoline can be non-covalently confined in silica through a simple sol gel method affording homogeneous spherical silica nanoparticles (NPs) (diameter 25 or 50 nm) stable with respect to the release of the metal in biological media and well suited for the development of MRI/optical multimodal tags.[23, 24] Furthermore, the non-covalent embedding of a mono-aqua Gd(III) complex in 25 nm silica nanoparticles leads to very high per-Gd relaxivity at 0.8 T due to the restricted motion of the complex in silica.[23] However, the non-covalent embedding of multi-aqua Gd(III) systems in silica nanoparticles and its influence on stability and relaxivity have not been investigated so far in spite of the potential interest of such system for application at high field MRI.

Here, we report the new tris-aqua Gd(III) complex $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ containing a tripodal hydroxyquinolinate ligand and its non-covalent incorporation in 25 nm silica nanoparticles. This complex shows high thermodynamic stability and high relaxivity. Moreover, its incorporation into silica nanoparticles leads to higher relaxivity, improved kinetic stability and suppression of anion binding.

The $H_3dhqN\text{-}SO_3$ ligand was synthetized in three steps from the commercial (2-methoxyphenyl)methanamine and from the 8-(benzyloxy)quinoline-2-carbaldehyde[25] with a 15 % total yield. The lanthanide complexes $[Ln(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ (Ln = Y, La, Lu) were prepared in situ by reacting the $H_3dhqN\text{-}SO_3$ ligand with the appropriate lanthanide chloride salt followed by adjustment of the pH to 8. The resulting complexes show high solubility in water (> 30 mM).



Scheme 1. Structure of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex.

The longitudinal relaxivity (r_1) of the complex $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ measured in water at pH 7.4 and at 298 K (Table 1) has high values of 12.23 and 10.65 $mM^{-1}s^{-1}$ at 0.8 and 4.7 T respectively. For comparison, the relaxivity of the commercial contrast agent *Magnevist*, the mono-aqua Gd(III) complex of DTPA,

is only 4.06 $mM^{-1}s^{-1}$ at 4.7 T. The relaxivity of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex in bovine serum (8.9 $mM^{-1}s^{-1}$ at 4.7 T), is twice as high as that of $[Gd(DTPA)(H_2O)]^{2-}$ measured in the same conditions (4.28 $mM^{-1}s^{-1}$). The NMRD profile of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ at 25°C and pH=7.4 in BSA (S6) shows a dramatic increase of the relaxivity at low field suggesting that the tumbling of the complex is slowed down by the presence of a non-covalent interaction with BSA as observed for other hydrophobic chelates.[3, 26] In contrast, a significant decrease of r_1 is observed when 50 equivalents of carbonate anion are added to a water solution of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ (7.02 $mM^{-1}s^{-1}$ at 4.7 T) suggesting that the carbonate anion partially displaces the water molecules from the Gd(III) coordination sphere.

Table 1. r_1 ($mM^{-1}s^{-1}$) values measured in water at pH = 7.4 and 310 K for the $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3}$ complexes and for $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs.

r_1 ($mM^{-1}s^{-1}$)	0.8T	1.2 T	2.3 T	4.7 T	9.4 T
$Gd(DTPA)(H_2O)]^{2-}$ *	3.73	3.34	3.22	2.87	2.72
$[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$*	9.44	9.25	9.01	8.49	6.88
Si25NPs	89±5	77±5	41±4	17±2	9±1

*Results reported as mean avec ±0.06 standard error

The values of the relaxivity measured for $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ in H_2O are consistent with those expected for a tris-aqua complex[26] (for example a value of 9.39 $mM^{-1}s^{-1}$ at 1.0 T , 298 K, and pH 7.4 was found[27] for the $[Gd(dpaa)(H_2O)_3]$[28] complex with the tripodal hexadentate picolinate ligand $dpaa^{3-}$). The presence of three water coordinated to the Gd(III) was confirmed by luminescence lifetime measurements of the $Eu(^5D_0)$ excited states on the analogous $[Eu(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex in H_2O and D_2O. The relaxivity of the tris-aqua dhqN-SO_3 complex is among the highest values previously reported for mononuclear Gd complexes of low-molecular weight.[9, 11]

The high relaxivity of this tris-aqua complex over a wide range of magnetic fields renders it attractive for potential application as an MRI contrast agent. However, for such an application high thermodynamic stability and high kinetic inertness are also required. Thus, the thermodynamic stability of the Gd and Zn complexes at pH 7.4 was investigated using UV-visible spectrophotometry by a competitive titration with H_5DTPA. The measured pGd (pGd = -log $[Gd_{aq}]_{free}$ for a total concentration of $[Gd]_{tot} = 10^{-6}$ M and $[L]_{tot} = 10^{-5}$ M at pH=7.4) for $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ has a high value of 18.9 for a q=3 compound. This indicates that the thermodynamic stability of this compound is comparable to that of *Magnevist*, (pGd = 19.1).[26] This is quite remarkable for a hexadentate ligand showing that the hydroxiquinolinate is a very effective binding group. The thermodynamic stability of the Zn(II) complex of dhqN-SO_3, calculated from experimental data, is much lower than that of the Gd(III) complex with a pZn at 15.0.

Moreover, we have evaluated the kinetic inertness of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex, a very important requirement for potential *in vivo* applications of Gd complexes. We reacted $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ with one equivalent of $ZnCl_2$ in phosphate buffer at pH 7.0 and the occurrence of transmetallation was monitored by relaxometry.[29] The replacement of Gd(III) by Zn(II) during the measurement leads to the precipitation of insoluble $GdPO_4$ and to the decrease of the relaxation rate R_1 of the solution. The time required to reach 80% of the initial R_1 value is a good estimate of the kinetic inertness. This time is of 10 min for $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$, a rather short value when compared to clinically used acyclic ligands such as $[Gd(DTPA\text{-}BMA)(H_2O)]$ (124 min) and $[Gd(DTPA)(H_2O)]^{2-}$ (383 min).

In order to prevent transmetallation and endogenous anion binding we incorporated the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex into silica NPs ($[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs). These nanoparticles were prepared by a reverse microemulsion procedure, based on the ammonia-catalyzed hydrolysis of tetraethyl orthosilicate (TEOS) in the presence of the complex.[23] Scanning Electron Microscopy (SEM) images of the particles confirmed that the obtained SiNPs are spherical and uniform in size with a diameter of 25 ±5 nm. The average number of Gd complexes was evaluated at 60 by NMR susceptibility shift measurements[30] on a weighted amount of dry nanoparticles suspended in water. Luminescence lifetime measurements of the $Eu(^5D_0)$ excited states on the analogous $[Eu(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs in H_2O and D_2O show that the hydration number of the complex is unchanged when embedded in the nanoparticles .

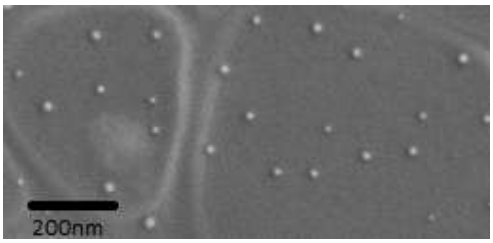


Figure 1. SEM image of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs.

The water proton longitudinal relaxivity r_1 due to $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs was measured at 298 and 310 K over a large range of resonance frequencies ν_I (field B_0 from 0.1 to 4.7 T) of the proton spin I and chosen values are given in Table 1.

The relaxivity of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs is very high at 0.8 T and 298K (90 $mM^{-1}s^{-1}$ per-Gd). Their NMRD profile (S7) is reminescent of the shape observed for slowly tumbling systems, with a maximum at 0.8 T and an abrupt drop of the relaxivity observed after 1.2 T. The high relaxivity per-Gd measured at 0.8 T is similar to the value measured in the same conditions for the mono-aqua complex [Gd(ebpatcn)(H2O)]@Si25NPs (84 $mM^{-1}s^{-1}$). [22] This increase of the relaxivity was explained in terms of the restricted mobility of the complexes confined in the silica matrix through electrostatic and hydrogen-bonding interactions and the reduced mobility of the water molecules in the silica pores.[23, 31] The relaxivity of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs is still very high at 1.2 T (78 $mM^{-1}s^{-1}$) and remain larger than that of the free complex at 4.7 T (18 $mM^{-1}s^{-1}$). Besides, since the average number of Gd complexes was evaluated at 60 by NMR susceptibility shift measurements,[30] the relaxivity on a per NP basis amounts to 5400 $mM^{-1}s^{-1}$ at 0.8 T and 1080 $mM^{-1}s^{-1}$ at 4.7 T. Preliminary studies show that a larger number of complexes (3880) can be confined in 55nm NPs leading to a per-NP relaxivity of 50440 $mM^{-1}s^{-1}$ at 4.7 T. Furthermore, monitoring of the relaxivity of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs after addition of one equivalent of $ZnCl_2$ (ESI) shows that the time required for R_1 to reach 80% of its initial value is 1000 min. This duration is significantly longer than that measured for the complex $[Gd(DTPA)(H_2O)]^{2-}$ (383 min).[9] Thus, the incorporation of a multi-aqua complex into silica NPs provides a convenient way to prevent transmetallation. We also found (Figure

3) that the relaxivity of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs measured in bovine serum at pH 7.4 , 4.7 T and 298 K amounts to 15 $mM^{-1}s^{-1}$. An analogue value (17 $mM^{-1}s^{-1}$) is measured in the same conditions in the presence of 50 equivalents of carbonate. These values show that endogenous anion binding is prevented by incorporation of the Gd(III) complex in silica NPs probably due to repulsive interactions which prevent the anions to go inside the NPs.

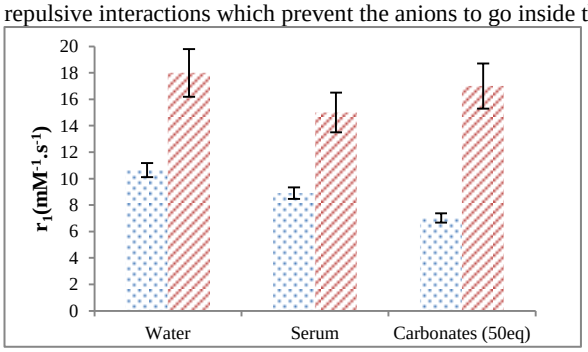


Figure 3. Relaxivity in different media of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ ( ) and $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs ( ) at 200MHz , pH 7.4 and 298K.

In conclusion, the hexadentate hydroxyquinoline based tripodal ligand dhqN-SO_3 affords a highly soluble tris-aqua Gd(III) complex with a thermodynamic stability comparable to that of contrast agents currently used in MRI examinations. The straightforward non-covalent embedding of this complex in silica nanoparticles leads to very high relaxivity at 1.2 T. Moreover, the incorporation of the complex into silica NPs renders the complex stable with respect to transmetallation by endogenous cations such as Zn(II) preventing the release of the toxic Gd(III) ion. Furthermore, endogenous anions do not bind to the Gd(III) ions of embedded complexes so that the relaxivity remains high in bovine serum at all fields. These results suggest that confinement of multi-aqua Gd(III) complexes in silica NPs is a promising route for the development of contrast agents with higher efficiency at all magnetic fields.

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Notes and references

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Electronic Supplementary Information (ESI) available: [Synthesis, and experimental details]. See DOI: 10.1039/c000000x/

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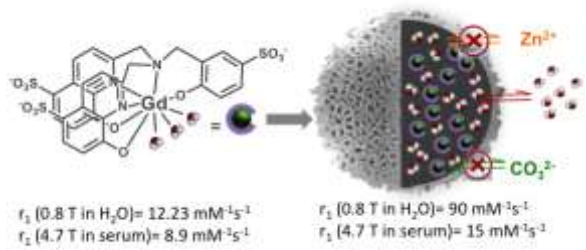

Confinement of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ in silica nanoparticles prevents transmetallation or anion binding, yielding high relaxivity over a range of magnetic fields.