Relaxivity Modulation in Gd-Functionalised Mesoporous Silicas.

Fabio Carniato, a Lorenzo Tei, b Walter Dastrù, c Leonardo Marchese a* and Mauro Botta b*

a Dipartimento di Scienze e Tecnologie Avanzate and Nano-SISTEMI Interdisciplinary Centre, Università del Piemonte Orientale “Amedeo Avogadro”, Via Bellini 25/G, 15100, Alessandria, Italy. Fax: +39-0131360250; Tel: +39-0131360262; E-mail: leonardo.marchese@mfn.unipmn.it

b Dipartimento di Scienze dell’ Ambiente e della Vita, Università del Piemonte Orientale “Amedeo Avogadro”, Via Bellini 25/G, 15100, Alessandria, Italy; E-mail: mauro.botta@mfn.unipmn.it

c Molecular Imaging Center, Facoltà di Farmacia, Università di Torino, Via Nizza 52, 10100, Torino, Italy.

Contents:

1. Synthesis
2. Characterization
3. Figures and Table
1. Synthesis

NMR spectra were recorded on a JEOL Eclipse Plus 400 (operating at 9.4 Tesla). ESI mass spectra were recorded on a Waters Micromass ZQ.

**Synthesis of GdL:**


ii) 1-(3-aza-9-carboxy-2,5-dioxononyl)-4,7,10-tris(tert-butyloxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (2): DOTAMA(t-BuO)3-en (0.409 g, 0.67 mmol) and succinic anhydride (0.070 g, 0.69 mmol) were placed in a roundbottom flask, dissolved in 30 ml of CH2Cl2 and stirred 20h at room temperature. The reaction mixture was then washed with water (3 x 20 ml), the organic phase was dried with Na2SO4 and the solvent was evaporated under reduced pressure to yield a pale yellow solid (0.383 g, 0.54 mmol, yield 80 %). 1H NMR (CDCl3, 400 MHz) δ = 8.18 (s, CONH, 1H); 7.95 (s, CONH, 1H); 4.13, 3.87 (s, NCNH2CO, 8H) 3.70-3.20 (m, CCH2ring, 16H); 3.00 (b, CCH2NH, 4H), 2.63, 2.56 (s, CH2CH2CO, 4H), 1.48, 1.45 (s, C(CH3)2, 27H); 13C NMR (CDCl3, 100 MHz) δ = 175.0, 173.9, 171.9, 166.3, 166.0 (Carboxyl and carboxyamide groups, 6C), 82.1 (C(CH3)3, 3C), 56.0, 55.8 (NCH2CO, 4C); 51.6, 48.8 (NCH2 ring, 8C); 39.5, 39.1 (CONHCH2, 2C); 37.5, 29.4 (CH2CH2COOH, 2C); 28.2, 28.0 (C(CH3)3, 9C). ESI-MS (m/z): (calcd. for C34H63N6O10 715.9), found 715.5 (M + H+).

iii) 1-(3-aza-9-carboxy-2,5-dioxononyl)-4,7,10-tris(carboxylmethyl)-1,4,7,10-tetraazacyclododecane (3): Compound (2) (0.383 g, 0.54 mmol) was dissolved in CH2Cl2 (15 mL) and trifluoroacetic acid (15 mL) and stirred at room temperature overnight. The solution was then evaporated in vacuum and the product was precipitated with excess of diethyl ether, isolated by centrifugation, washed thoroughly with diethyl ether and dried in vacuo obtaining pure desired product as amorphous white solid. (0.257 g, 0.47 mmol, yield 87.6%). 1H NMR (D2O, 400 MHz): δ = 3.82, 3.73 (s, NCH2CO, 8H), 3.62-3.27 and 3.20-2.90 (m, CCH2ring and CH2NH 20H), 2.63, 2.54 (t, J = 7.0 Hz, CH2CH2COOH, 4H). 13C NMR (D2O, 100 MHz): δ = 177.8, 170.1, 163.4, 163.0 (Carboxyl and carboxyamide groups, 6C), 56.6, 55.8, 51.7 (NCH2CO, 4C), 51.0, 48.3 (NCH2 ring, 8C), 38.9, 38.6 (CONHCH2, 2C), 31.2, 29.5 (CH2CH2COOH, 2C). ESI-MS (m/z): (calcld. for C34H63N6O10 547.6), found 547.4 (M + H+). The final compound contains, as an impurity, ca. 5 - 10% of the un-functionalized ligand (DOTAMA-En) bearing a primary amino group. We did not purify further the product at this stage since this impurity does
not react during the following steps and can be removed by simple filtration after the grafting procedure on the mesoporous silica particles.

iv) GdL:

Compound (3) (0.55 g, 1.0 mmol) was dissolved in 30 ml of H2O. 0.371 g (1.0 mmol) of GdCl3.6H2O was added to the solution. The pH of reaction was corrected to 6.5 by addition of few ml of diluted NaOH solution. The mixture was stirred at room temperature for 20h. Finally, unchelated Gd3+ ions were eliminated by precipitation of the hydroxide at basic pH by adding aliquots of a concentrated NaOH solution. The solvent was removed under reduced pressure leading to the formation of the desired product. ESI-MS (m/z): (calcd. for C22H34GdN6O10 700.2), found 700.5 (M – H+); correct isotopic distribution.

Scheme of GdL synthesis

Synthesis of mesoporous supports:

i) MCM-41 and SBA-15:


ii) NH2/MCM-41 and NH2/SBA-15:

1.0 g of calcined SBA-15 and MCM-41 was firstly treated under vacuum at 250°C for 2h in order to remove completely the water adsorbed on the silica surface, then both materials were suspended in toluene (100 ml) under nitrogen flow. 3-Aminopropyltriethoxysilane (99%, Sigma Aldrich) (40
wt%) was added to the suspensions. The reaction was carried on at 50°C under stirring for 20h. Finally, the products were filtered and washed several times with diethyl ether in order to remove the un-reacted silane. The samples were dried at 60°C for 2h.

**Synthesis of GdL/SBA-15 and GdL/MCM-41:**

**Anchoring of GdL on functionalized SBA-15 and MCM-41.**

700 mg of NH$_2$/MCM-41 (or NH$_2$/SBA-15) were suspended in 50 ml of DMF (Sigma Aldrich) for 30 min. In parallel, GdL (0.500 g, 0.7 mmol) and 0.27 g (0.7 mmol) of O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (Sigma Aldrich) were dissolved in 40ml of DMF. The solution was added to the suspensions of NH$_2$/MCM-41 (or NH$_2$/SBA-15) and stirred at room temperature. The reactions were carried on under basic conditions, in the presence of N,N-Diisopropylethylamine (124µl) at 50°C for 24h. Finally, the solutions were filtered and washed several times with water. The products were dried at 60°C for 2h to obtain white powders.

**Quantification of Gd-loadings per particle of GdL/MCM-41 and GdL/SBA-15.**

The determination of Gd-loading per each silica particle was calculated considering the particles as spheres with average diameter 30 nm (for GdL/MCM-41) and 600 nm (for GdL/SBA-15) and considering the densities of GdL/MCM-41 and GdL/SBA-15 around 0.8 g/cm$^3$ (Karen J. Edler, Philip A. Reynolds, John W. White and David Cookson, *J. Chem. Soc., Faraday Trans.*, 1997, 93 (1), 199-202) and, 2.2 g/cm$^3$ (Mietek Jaroniec and Leonid A. Solovyov, *Chem. Commun.*, 2006, 2242–2244), respectively.
2. Characterization

- Infrared (IR) spectra of GdL/SBA-15 and GdL/MCM-41 and the pristine porous supports have been recorded in the range 4000 - 400 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution using a Bruker Equinox 55 spectrometer. The samples in the form of self-supported pellets have been outgassed (in vacuum (10^-4 mbar) for 2h.

- High resolution transmission electron microscopy (HRTEM) images were collected on a JEOL 3010 microscope operating at 300 kV. Specimens were prepared by dispersing the sample by sonication in isopropanol and by depositing few drops of the suspension on carbon-coated grids.

- N\(_2\) physisorption measurements were carried out at 77K in the relative pressure range from 1*10^-6 to 1 P/P\(_0\) by using a Quantachrome Autosorb 1MP/TCD instrument. Prior to the analysis the samples were outgassed at 373K for 3 h (residual pressure lower than 10^-6 mbar). Apparent surface areas were determined by using Brunauer-Emmett-Teller equation, in the relative pressure range from 0.01 to 0.1 P/P\(_0\). Pore size distributions were obtained by applying the NLDFT method (N\(_2\) silica kernel based on a cylindrical pore model applied to the desorption branch).

- Thermogravimetric analyses (TGA) of materials were performed under Argon flow (20 ml/min) with a SETSYS Evolution TGA-DTA/DSC thermobalance, heating from 50 to 800°C at 10 °C/min.

- The observed longitudinal water proton relaxation times (T\(_{obs}\)) were measured on a Stelar Spinmaster spectrometer (Stelar, Mede (PV) Italy) operating at 20 MHz, by means of the standard inversion-recovery technique (16 experiments, 2 scans). The tenor of Gd(III) in the final materials was estimated by elemental analysis (ICP/MS) by the NEOSIS S.A.S Lab. in Turin, Italy.

- MR images were obtained on a 3T Bruker spectrometer equipped with a microimaging probe. The experimental details are reported in the paper.
3. Figures and Table

Figure 1: IR spectra in vacuum of A): SBA-15 (a), NH$_2$/SBA-15 (b) and GdL/SBA-15 (c) and B): MCM-41 (a), NH$_2$/MCM-41 (b) and GdL/MCM-41 (c).

Figure 2: Thermogravimetric analysis (TGA) of GdL/SBA-15 (curve a) and GdL/MCM-41 (curve b) collected under Argon flow at 10°C/min.
Table 1: Textural parameters of mesoporous supports and the hybrid materials.

<table>
<thead>
<tr>
<th></th>
<th>SBET $\text{m}^2/\text{g}$</th>
<th>Pore Volume $\text{cm}^3/\text{g}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA-15</td>
<td>750</td>
<td>0.94</td>
</tr>
<tr>
<td>NH$_2$/SBA-15</td>
<td>535</td>
<td>0.70</td>
</tr>
<tr>
<td>GdL/SBA-15</td>
<td>530</td>
<td>0.78</td>
</tr>
<tr>
<td>MCM-41</td>
<td>890</td>
<td>1.47</td>
</tr>
<tr>
<td>NH$_2$/MCM-41</td>
<td>855</td>
<td>1.24</td>
</tr>
<tr>
<td>GdL/MCM-41</td>
<td>625</td>
<td>0.95</td>
</tr>
</tbody>
</table>