Supplementary Material (ESI) for Chemical Communications

MRI contrast agent delivery using spore capsules: controlled release in blood plasma

Experimental data

Materials

Lycopodium clavatum was purchased from Tibrewala International (Katmandu, Nepal). Omniscan was purchased from GE Healthcare. All other reagents were purchased from Sigma-Aldrich or Fisher Scientific UK Ltd. and were of general purpose grade unless otherwise stated.

Extraction of sporopollenin from *Lycopodium clavatum*

Raw *Lycopodium clavatum* spores (200.5 g) were suspended in acetone (900 ml) and stirred at 60 °C for 4 hours. The spores were recovered by filtration through a sintered glass funnel (porosity grade 3) and washed with acetone (250 ml).

The spores were suspended in 6 % KOH (54.0 g, 900 ml) and stirred at 80 °C for 6 hours. The spores were recovered by filtration through a sintered glass funnel (porosity grade 3) and washed with hot H_2O (500 ml x 2) and re-suspended in fresh 6 % KOH and stirred at 80 °C for a further 6 hours. The spores were recovered by filtration through a sintered glass funnel (porosity grade 3) and washed with hot H_2O (500 ml x 6).

The spores were then suspended in 85 % *ortho*-phosphoric acid (900 ml) and stirred at 60 °C for 5 days. The spores were recovered by filtration through a sintered glass funnel (porosity grade 3) and washed with hot H_2O (500 ml x 2), 2 M NaOH (250 ml x 2), hot H_2O (500 ml x 6), PBS (250 ml x2), hot H_2O (500 ml x 2) and heated at 80 °C for 4 hours in ethanol (900 ml). The spores were recovered by filtration through a sintered glass funnel (porosity grade 3), washed with EtOH (500 ml) and then sonicated for 30 minutes at 40 °C in acetone (500 ml). The spores were recovered by filtration through a sintered glass funnel (porosity grade 3), washed with acetone (500 ml) and then left to dry in air for several hours before drying further in an oven at 60 °C until constant weight.

Encapsulation of contrast agent

Sporopollenin (2.0 g) was mixed with the aqueous contrast agent solution (Omniscan, 0.5 M, 2 mL), stirred for 1 minute and then subjected to a vacuum for 2 hours. The spores were then washed with 6 x 10 ml deionised water, on a porosity grade 3 sintered glass frit, to remove any contrast agent that was not encapsulated within sporopollenin. The control was treated in the same way using either PBS or deionised water.

Gadolinium (CA) loading level

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) was performed on a Perkin Elmer Optima 5300DV Spectrometer by Robert Knight, Department of Chemistry, University of Hull, and blank subtracted. The optimum emission wavelength was selected for each element and the parts per million (ppm) were calculated. The sample was prepared as described above, it was dried by air suction and so considerable water encapsulation is still expected. The concentration of gadolinium in the sample was found to be 0.219% by mass which equates to 0.799% of contrast agent by mass. This represents a 32-fold dilution by mass based on the original 0.5 M Omniscan scan solution (density 1.15 gcm^{-3}).

Scanning Electron Microscopy

Scanning electron microscopy images were obtained using a Leica Cambridge Stereoscan 360 Scanning Electron Microscope. We thank Tony Sinclair, Department of Chemistry, University of Hull for collecting the SEM images. To analyse the distribution of the gadolinium, elemental contrast images of the CA encapsulated spores were recorded without coating (imaged at 35 Pa), see Fig. 1, as well as carbon coated samples which were imaged normally under high vacuum. The images were obtained using either backscattered electrons to produce elemental contrast, the others being normal topographical contrast. EDX spectrometry detected gadolinium(III) both on the bright areas as well as away from the bright areas. This uniform low distribution of the gadolinium agent is consistent with encapsulation of an aqueous solution of the contrast agent. When metal salts are deposited on the surface of the spores there is clearly detectable localisation at similar concentration levels.

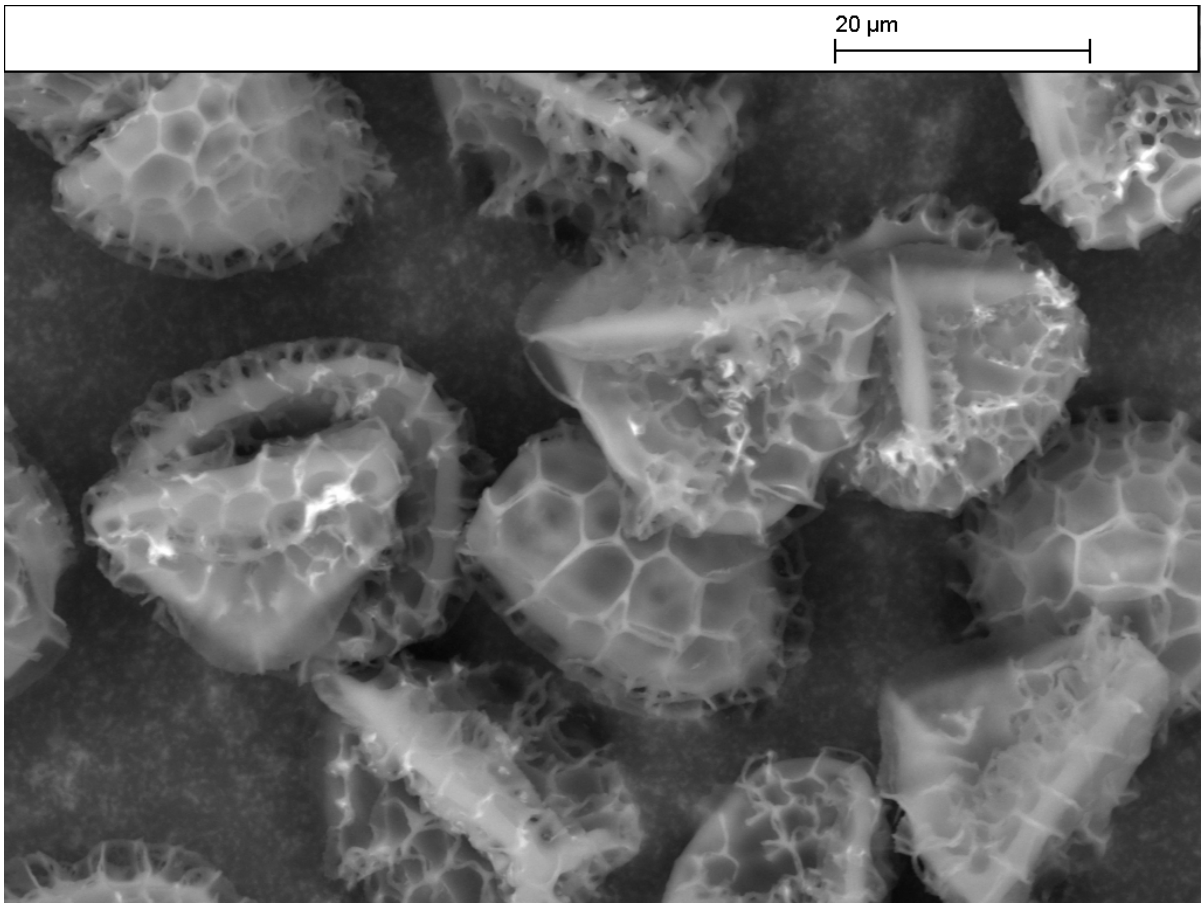

Figure 1: Elemental contrast SEM image of the gadolinium(III) CA encapsulated spores.

Digestion of spores in plasma

Sporopollenin (1.0 g) was suspended in human plasma (4.0 ml) and incubated at 37 °C for 30 minutes. The sporopollenin was then collected by centrifugation (3000 rpm, 10 minutes) and washed with water, ethanol, acetone and left to dry in open air then dried to constant weight in a freeze-dryer.

Detection of the intact CA in plasma using mass spectrometry

Mass spectrometry experiments were carried out using a Varian 500-MS LC by electrospray ionisation, see Figs. 2 and 3. Detection of the intact agent from a plasma sample was carried out with a sample that had been digested in plasma for 48 hours, then filtered to remove the spores followed by filtration through a 0.1 μm syringe filter. The sample was prepared for analysis by adding 300 μl of filtered plasma to 900 μl of acetonitrile to precipitate the proteins. The intact CA is clearly observed at 574 a.m.u.

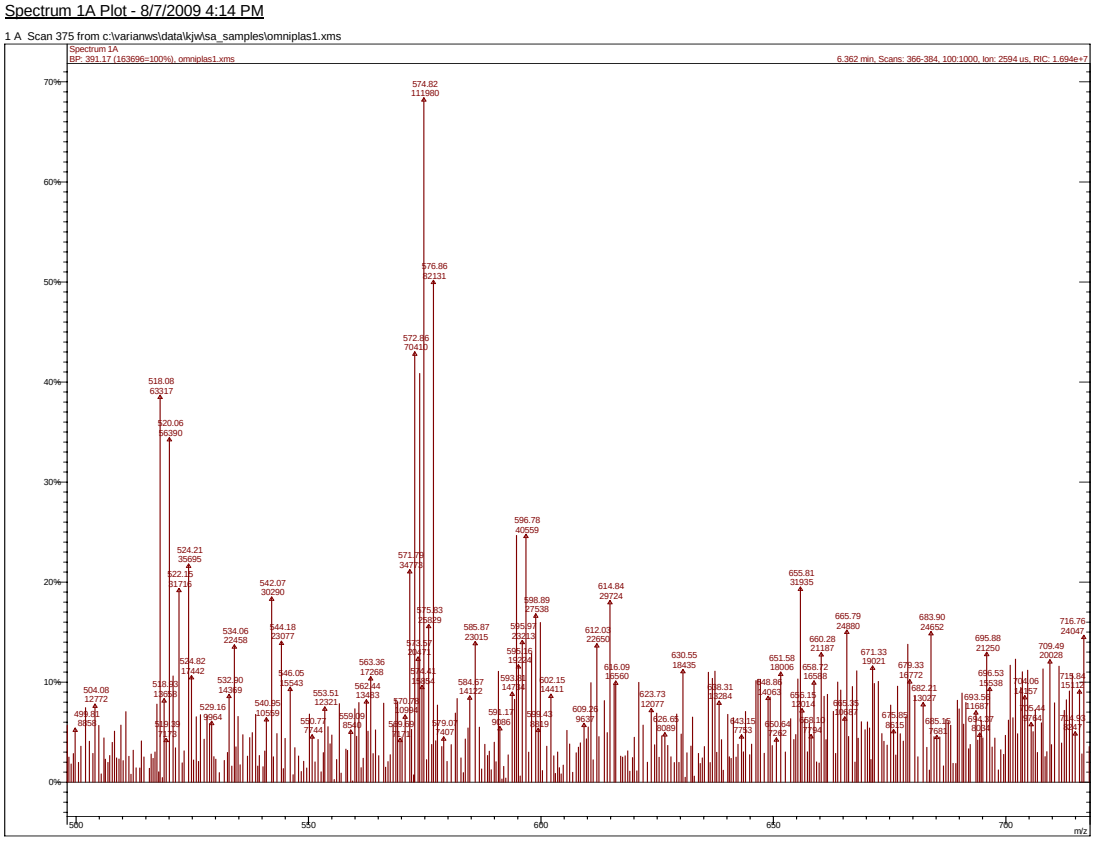

Figure 2: Electrospray mass spectrum of the digested spores to show release of the intact encapsulated CA.

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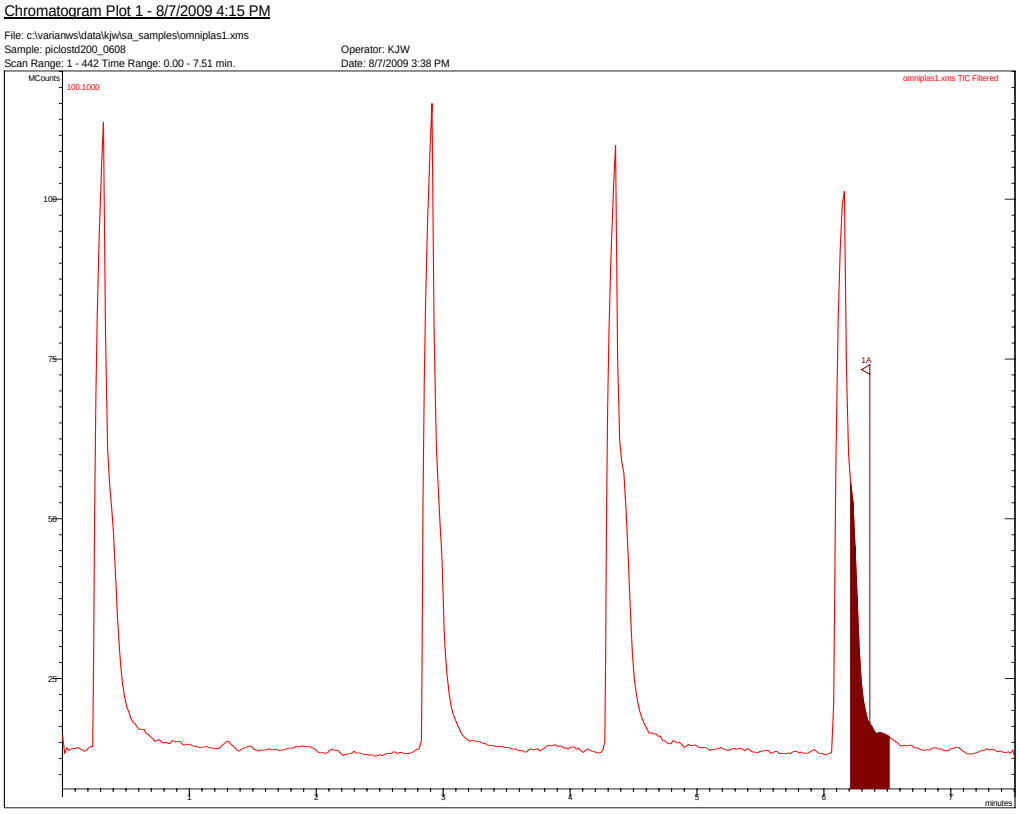

Figure 3: LC traces of four separate sample injections (the CA was detected in all four spectra).

MRI methods

All images were collected on a Bruker AVANCE II widebore 11.74 T magnet fitted with a Bruker microimaging probe with a 10 mm birdcage coil or a 2 mm solenoid coil. Standard pulse sequences were used from the Bruker Paravision library.

3D spin echo images were acquired using the solenoid coil and Bruker's m_se3d pulse sequence. The 90^{o} pulses length was 6µs, T_E = 3.525 ms, T_R = 70 ms, SW = 101010.1 Hz. The matrix dimension was 256 x 128 x 128, the FOV was 6 mm x 0.19 mm x 0 .19 mm and a voxel size was 23 µm x 15 µm x 15 µm. 8 acquisitions were used per increment giving a total measuring time of 152 minutes.

T1 weighted images were acquired using the 10mm birdcage coil and Bruker's FLASH pulse sequence with a 1ms 30^{o} pulse, T_E = 6 ms and TR = 80 ms, SW = 5400 Hz at 30^{o}C, see Figure 4. The matrix dimension was 128 x 128, the FOV was 15 mm x 15 mm and a voxel was 0.117 mm x 0.117 mm. The slice was 1mm thick. 1 scan was taken per increment giving a total measuring time of 10s.

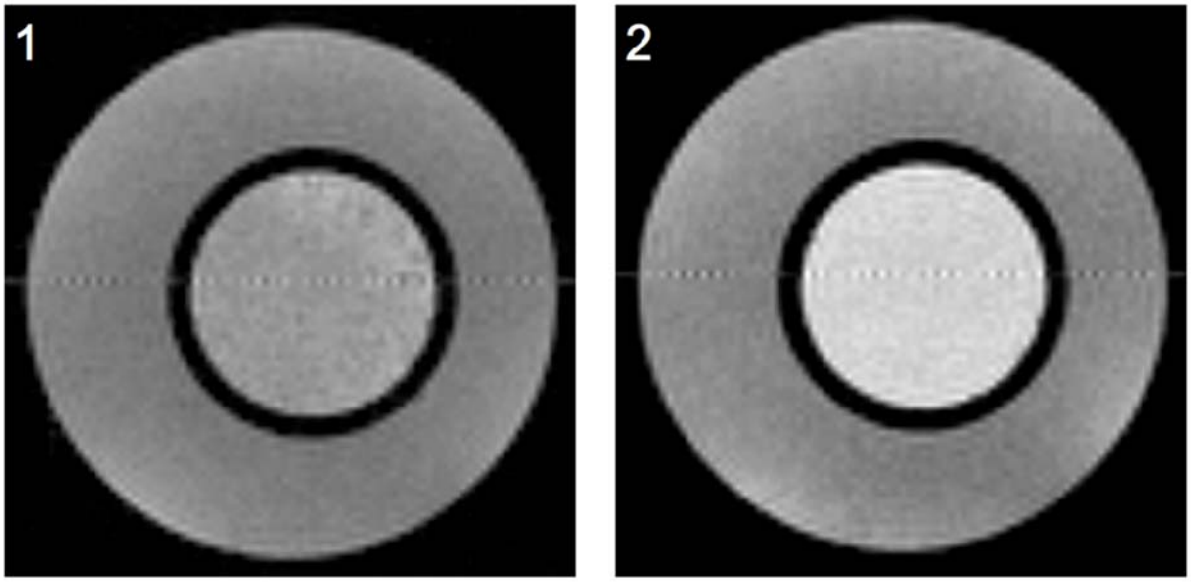

Figure 4: T1 weighted images of the 2 tube experiments. The inner tube contains spores loaded with Omniscan and blood plasma. The outer tube contains blood plasma only. The diameter of the outer tube is 10mm. Cross sections were taken approximately 10mm above the spore pellet. The 2 images shown were collected at T = 0 and T = 8 hrs.