Supplementary material (ESI) for Journal of Materials Chemistry

Bacteria encapsulation in a magnetic sol-gel matrix

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Electronic Supplementary Informations

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ESI-1 : Experimental details

Bacterial strain and growth conditions

Stock cultures of cells were prepared from cultures stored at -80 °C in Luria-Bertani (LB) broth supplemented with glycerol. When needed, cultures of bacteria in LB broth at 37 °C were prepared overnight. An inoculum (0.1 mL) was added to a glucose (20 mM)-minimum phosphate broth (minimum medium MM) (50 mL) at pH 7 in a flask (250 mL) and grown at 37 °C under stirring (200 rpm). After 3 h of incubation, corresponding to the mid-exponential phase of growth, the culture was harvested by centrifugation at 6000 rpm for 15 min at 9 °C. The pellet was washed twice with phosphate buffer (PB) (100 mM), and diluted to reach a concentration of 10^9 cells mL-1 in PB (working cell suspension, WCS) or in 10% w/w glycerol PB (gly-WCS).

Growth of bacteria was studied in a MM solution. In a typical experiment, a bacteria inoculum (100 µL) was added to the culture medium MM (50 mL) in a flask (250 mL). In order to study their effect, ferrihydrite particles, at different concentrations (5, 2.5 and 0.5 g.L-1), were added into the MM. Cells were incubated at 37 °C under stirring. The growth of bacteria was followed by turbidity measurements; the optical density (OD) of suspension aliquots recorded at λ = 600 nm is proportional to bacteria concentration.

Preparation of iron hydroxide sol

The iron hydroxide particles are obtained by alkalinisation of a chloride solution iron (III) (0.05 M) by a solution of sodium hydroxide (1 M). The pH is adjusted to a value equal to 7. The final concentration of iron is 0.04 $mol.L^{-1}$. The gelatinous precipitate is recovered by centrifugation. In order to remove the chloride anions and to reduce the ionic strength, nanoparticles were washed several times until it could not be detect, by adding $Ag(NO)_3$, to the supernatant.

A vigorous shaking of 2 g of the iron hydroxide gelatinous precipitate leads to the particle resuspension forming a concentrated sol. The addition of 0.5 mL phosphate

buffer solution (TP or TP-Gly) led to the gel formation. The synthesized gel is brown and opaque.

Encapsulation of cells and references

The gel was formed by adding PB or WCS solution (0.5 mL) in the iron hydroxide sol (BS) previously prepared. The mixture was homogenized under gentle stirring (300 rpm). Gelation occurred instantaneously at room temperature. Wet gels were aged for 1 hour and 1, 15 or 30 days at 20 °C in the mother solution in a closed flask.

Viability measurements

Wet gels were crushed, vigorously stirred with phosphate buffer (3 mL) for 1 hour after one, two and four weeks. A series of 10-fold dilutions of WCS or resuspended gels (0.1 ml) diluted in PB (0.9 ml) were surface-plated in triplicate on LB-agar. Plates were then incubated at 37 °C for 24 h. The colony forming units count after one day is taken as a 100% reference.

Gel characterization

Cells containing aerogels were also obtained by supercritical drying in CO_2 after fixing the samples (ou ferrihydrite gel) in 2.5% glutaraldehyde and dehydration through ethanol baths (30%, 50%, 70%, 80%, 90%, 95% and 100%). The supercritical drying process was performed on a BAL-TEC 030.

Then they were coated with gold in a Balzers Union SCD 40 sputter-coater and studied by scanning electron microscopy (SEM) using a JEOL model JSM-5510 at an accelerating voltage of 10 kV.

The aged wet iron hydroxide gels containing cells were fixed in 2.5% glutaraldehyde and postfixed in 2% osmium tetraoxide. The samples were then dehydrated through successive ethanol baths (50%, 70%, 95%, 100%) prior to inclusion in araldite. An ultra

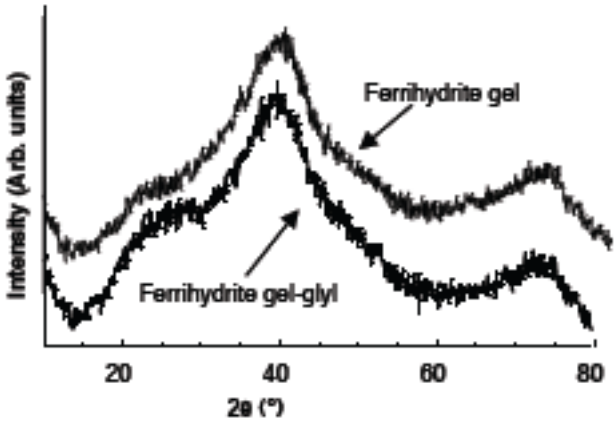
microtome (Ultracut Reichert Jung) was used to section the block into 70 nm thin sections that were stained with phosphotungstic acid for electron microscopy. Observation of ultra thin sections by transmission electron microscopy was performed on a Philips CM12 electron microscope, operated at 120 kV.

The porosity of aerogels was measured by nitrogen sorption experiments performed at 77 K on a Micromeritics 2010 sorptometer. Prior to analysis, samples were first degassed at 60 °C under a 3 μm Hg pressure. Specific surface areas SBET were determined by the Brunauer–Emmett–Teller (BET) method in the relative pressure range of 0.05–0.3.

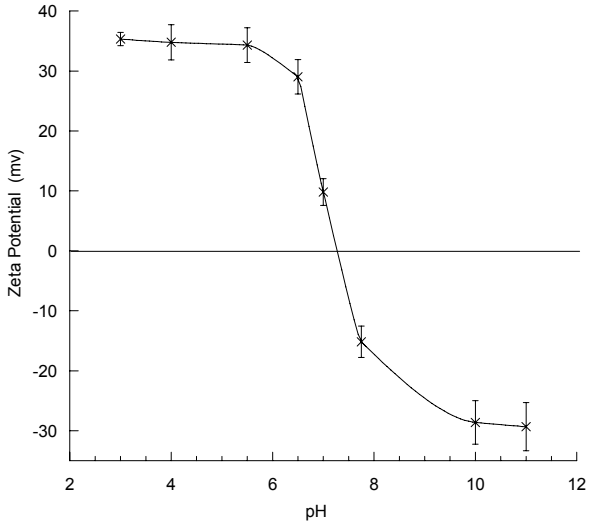
The crystalline structure of the aerogels was studied by X-ray diffraction (XRD) on a Panalytical X'pert Pro diffractometer equipped with a multichannel X'celerator detector, using Co Kα radiation (λ = 1.7889 Å) in the 2θ range of 10–80°.

A Quantum Design MPMS-5S super quanducting interference device (SQUID) magnetometer was used for magnetic characterization in the 2–300 K temperature range. The thermal zero-field cooling (ZFC) and field cooling (FC) susceptibility, χ, variations were measured in a magnetic field of 500 Oe. To highlight the existence of an exchange bias in some samples, a measurement of the isothermal magnetization at 2 K, after cooling from room temperature, under a field of 500 kOe, was also performed.

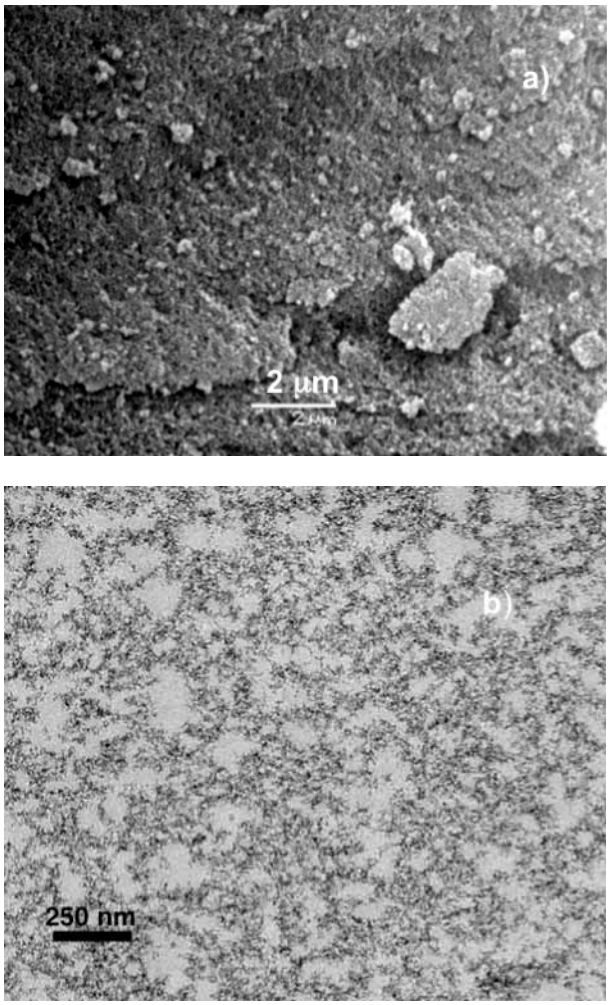
The electrophoretic mobility of the particles was measured at room temperature with an electrophoresis light scattering Zeta Plus instrument (Brookhaven Instruments Corp). The measurement is performed on particle suspensions in a 25 $g.L^{-1}$ concentration and a constant ionic strength I = 0,1.



ESI-2 : XRD diagrams of ferrihydrite gel containing or not glycerol



ESI-3 : Zeta potential of ferrihydrite colloids in a 0.1 M NaCl aqueous solution as a function of pH



ESI-4 : SEM (a) and TEM (b) images of ferrihydrite gels obtained in the absence of glycerol