Supplementary Material (ESI) for Chemical Communications

Highly Active Horseradish Peroxidase Immobilized in 1-Butyl-3-methylimidazolium Tetrafluoroborate Room-Temperature Ionic Liquid based Sol-Gel Host Materials

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EIS

Experimental details

Chemicals

$BMIM^+BF_4^-$ was purchased from Acros Organics and was dried in vacuum at 60 ^oC for 24 h. Horseradish peroxidase (HRP, RZ≈3.0, 250 U mg^{-1}) was purchased from BioBasic Incorporation. Tetraethyl orthosilicate (TEOS) was obtained from Sigma (≥ 99%). Guaiacol (≥ 99%, chemical purity) was purchased form Shanghai Chemical Regent Corporation. HCl, H_2O_2 (33%) and sodium phosphates were chemical purity and purchased from Beijing Chemical Regent Corporation. H_2O_2 was titrated by $KMnO_4$ before the activity assay. All the reagents and chemicals were used as received without further purification.

Preparation of HRP-IL@GEL

To synthesis the sol containing $BMIM^+BF_4^-$, 2 ml of TEOS, 1 ml of H_2O, 0.05 ml of 0.1 M HCl and 1 ml $BMIM^+BF_4^-$ were mixed in a beaker under magnetic stirring at room temperature. After 3 h, a homogenous sol was gotten. The gels were produced

by adding 0.4 ml of 2 mg/ml HRP (in 0.025 M phosphate buffer, pH 6.86) in 0.4 ml of sols with or without IL. The mixtures were sealed with paraffin film and placed at room temperature until gelation occurred. After 6 h, the film was pinned with many holes to evaporate the solvents and byproducts in the sol-gel reaction. The samples were put in vacuum oven and evacuated for over 3 days until a constant weight was gotten and no weight lost was observed below 100 ^{o}C in TGA spectra (Fig. S2). 0.1 g (without IL) and 0.19 g (IL) transparent monoliths were obtained respectively. No crack was found in the HRP-IL@GEL, on the contrary there were obvious cracks in HRP@GEL. The IL based silica gel was promised to be a good matrix of sensor. The dry gels were crushed into fine powders with a mortar and stored in refrigerator.

Characterization

TGA was carried out using a Perkin-Elmer 7 series TGA system. Measurements were conducted by heating the sample from 20 °C to 700 °C at a heating rate of 10 °C/min under air atmosphere.

FT-IR was conducted using a FTS135 infrared spectroscope (BIO-RAD, USA). FT-IR spectra (4000-450 cm^{-1}) of both as-synthesized and calcined samples were obtained by forming thin transparent KBr pellet containing the samples. Two typical peaks at ~ 1107 cm^{-1} (*) and 815 cm^{-1} (+) in the infrared spectra of both as-synthesized and calcined gels, corresponding to Si-O-Si asymmetric stretching vibration and O-Si-O vibration respectively, indicated the formation of SiO_2 framework.

SEM measurements were made on a XL30 ESEM FEG scanning electron microscopy

at an accelerating voltage of 20 KV. The TEM graph and diffraction pattern were taken with a JEOL-JEM-2010 operating at 200 kV (JEOL, Japan). Samples for TEM were prepared by dropping a diluted suspension of silica powder onto a standard carbon-coated (20-30 nm) formvar film on a copper grid (230 mesh).

BET surface and pore volume were measured on a Quantachrome NOVA 1000 Ver. 6.11 system at 77.4 K. All samples were first degassed in a vacuum at 200 °C for 2 h before analysis. The BET specific area was calculated from the nitrogen adsorption data in the relative pressure range from 0.01 to 0.3. The total volume was estimated from the amount adsorbed at a relative pressure of about 0.99. The average BJH pore diameter was calculated by using a NOVA Enhanced Data Reduction Software (Ver. 2.13)

UV-vis experiments were performed with a Cary 500 UV-vis spectrophotometer (VARIAN, USA).

Activity assay of immobilized HRP

In a general procedure, an exact amount of sample powders (3 mg of HRP@GEL, and 5.7 mg of HRP-IL@GEL, each containing 2.4×10^{-2} mg HRP) was weighed in a tube, then phosphate buffer (pH 6.86) was added to wash the samples at a vibrator. After 1 h, the washing solution was separated by decantation after centrifugation. The activity assay for the immobilized HRP was performed by measuring the initial oxidation rate of guaiacol with H_2O_2. The initial rate was obtained by measuring the absorbance changes at 470 nm of the maximum absorbance of the oxidation product of

guaiacol and the absorption coefficient of the oxidation product was 2.66×10^4 M^{-1} cm^{-1}. For the measurements of K_M and k_{cat}, constant amount of H_2O_2 (1.5 mM of final concentration) was added in a 1 cm photo-path length quartz cell with quantitative immobilized HRP and fixed concentration of substrate to a constant volume of solution (3 mL) in 0.025 M phosphate buffer (pH 6.86).

Before the addition of H_2O_2, the mixed solution was allowed to be equilibration for 15 min. The absorbance was scanned from 350 nm to 700 nm for 60 min with the interval of 2 min. For the assays of immobilized HRP, the initial rate of product formation (V_0) was evaluated from the points after 10 min where a linear relationship was gotten.

The K_M was calculated by constructing double reciprocal plots (Lineweaver-Burk equation) relating V_0^{-1} to S^{-1} ([substrate concentration]$^{-1}$), and fitting these to a linear model. k_{cat} was calculated by followed equation:

$$k_{cat}=V_{max}/[E]_{tol}$$

V_{max} is the maximum velocity of the enzyme reaction, which can be obtained from Michealis-Menten equation. $[E]_{tol}$ was the concentration of HRP in the reaction solution.

Thermal stability of the encapsulated HRP was examined from the retaining activities after the treatments at each temperature for 30 min, in comparison with the activities of the samples without the thermal treatment. The activity assay was the same as before.

Fig. S1 The IR image of the as-synthesized (a) and the calcined (b) HRP-IL@GEL.

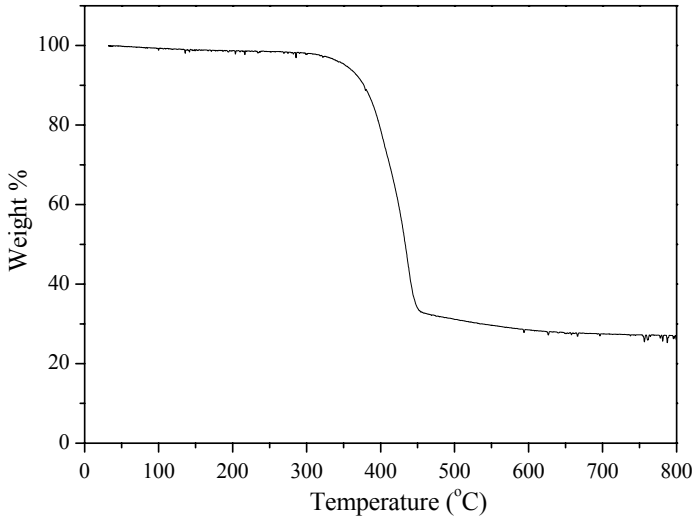

Fig. S2 The TGA of the as-synthesized HRP-IL@GEL.

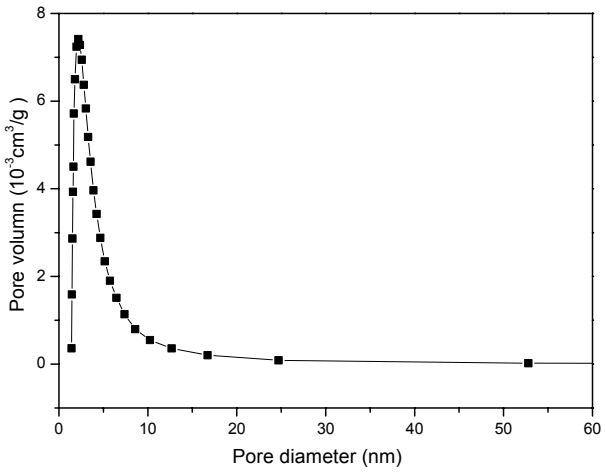

Fig. S3 The BJH desoption pore size distribution of the HRP-IL@GEL matrix after calcination.