ESI FOR:

Sub-100 nm TiO_2 Mesocrystalline Assemblies with Mesopores: Preparation, Characterization, Enzyme Immobilization and Photocatalytic Properties

Pedro Tartaj*

[a] *Instituto de Ciencia de Materiales de Madrid (CSIC), Cantoblanco, 28049, Madrid, Spain,;*

E-mail: ptartaj@icmm.csic.es

Experimental Section

Chemicals: All chemicals reactants were purchased from Sigma Aldrich except lysozyme that was obtained from Fluka. The reactants were used without additional purification. $TiOSO_4$ (1.25 M solution in H_2SO_4) was used as precursor of TiO_2. $TiCl_4$ was used as precursor of the anatase nanoseeds. Igepal CO-520 and cyclohexane dehydrated with zeolites were used as the surfactants and the continuous oil phase for the preparation of microemulsions. Deionized water was used in all experiments. Lysozyme from chicken egg white (70000 units/mg) was obtained from Fluka.

Preparation of anatase nanoseeds: Anatase nanoseeds of about 12 nm were obtained by the low temperature hydrolysis of $TiCl_4$ (N. Serpone, D. Lawless and R. Khairutdinov, *J. Phys. Chem.* 1995, **99**, 16646). Briefly 5.2 mL of $TiCl_4$ was slowly added to 200 mL of water maintained at 4 ºC under vigorous stirring. Following dialysis a colloidal sol containing 0.2 M of TiO_2 nanoseeds at pH = 3 was obtained.

Preparation of water-in-oil microemulsions: In a typical experiment 0.5 L of a water-in-oil microemulsion was prepared at room temperature by mixing and strong stirring a mixture of surfactant (0.2 M) with cyclohexane (0.5 L) and adding the adequate volume of $TiOSO_4$ (1.25 M) and H_2O. The methodology established for efficient preparation of the microemulsion was the following: All the surfactant was added to 0.25 L of cyclohexane (half of the volume) under magnetic stirring. After 5 min, an aliquot (1/4 of the total $TiOSO_4$ and H_2O) was added and the resulting mixture was stirred for about 15 min. This process was repeated until all the $TiOSO_4$ and H_2O were added. After this, the rest of the cyclohexane was added (0.25 L) and the microemulsion was stirred 1 h longer.

Preparation of mesocrystalline structures with spherical shape, mesoporosity and sizes below 100 nm: A summary of the experimental conditions used to prepare the samples is shown in Table S1. Once the microemulsion was formed (0.5 L as above-mentioned) the adequate volume of the sol containing TiO_2 nanoseeds (0.2 M) was added to the microemulsion. The resulting suspension was stirred for about 2 h and later introduced in an oven at 60 ºC. After 5 or 10 h depending on the $TiOSO_4$ concentration (see Table S1) the temperature of the oven was finally set to 80 or 120 ºC. Samples prepared at 80 ºC remained at this temperature for 72 h. For samples prepared at 120 ºC the temperature program consisted in raising the temperature at steps of 20 ºC (80, 100 and finally 120 ºC) remaining 24 h at each temperature. This process was carried out to avoid abrupt changes in temperature (and so pressure). Then, the resulting material was washed with EtOH several times, dried at 50 ºC and heated at 300 ºC/24 h to eliminate the interstitial surfactant and so generate mesoporosity. By using this methodology we were able to obtain in single experiments as much as 1 or 2 g (depending on the initial amount of $TiOSO_4$) of material. It is important to mention that the residence time at 60 ºC (time needed to hydrolyse 20-30 wt% of $TiOSO_4$ expressed in TiO_2 content) for each reactants concentration was determined for total volumes of about 10 mL (instead the 500 mL used for reactions).

Characterization techniques: Phase identification was performed by X-ray analysis. X-ray diffraction (XRD) patterns were collected from 5 to 70º (2θ) by using a Bruker D8 Advance instrument with CuKα radiation and a SOLX detector operating at 40 kV and 30 mA. The crystal domain size was determined from the X-ray profiles following the Scherrer equation by using the DIFRACPLUS EVA software (BRUKER AXS). The morphology, particle size and crystallinity of the materials were examined by transmission electron microscopy (TEM, 2000 FX2, JEOL) and high-resolution transmission electron microscopy (HR-TEM, 300, JEOL). The mean size (X), the standard deviation (SD) and the polydispersity index (defined

as *SD*/*X*) were evaluated from the electron micrographs by counting around 100 particles. Nitrogen adsorption and desorption isotherms were performed at –196ºC in a Micromeritics ASAP 2010 volumetric adsorption system. The BET surface area was deduced from the analysis of the isotherm in the relative pressure range from 0.04 to 0.20. Pore size distributions were estimated using the BJH model.

Lysozyme Immobilization: Around 10 mg of the samples was dispersed at room temperature in 10 mL of lysozyme solution (initial concentration of lysozyme: 1 mg mL^{-1}). The immobilization experiments were carried out under magnetic stirring in a closed vessel at pH of 10 (buffer 0.025M $NaHCO_3$ + 0.025M Na_2CO_3). To evaluate the amount of enzyme immobilized, we periodically monitored the concentration of lysozyme in the solution by means of a UV-vis spectrophotometer (Perkin Elmer Lambda 35) using UV absorption at 280 nm. Prior to recording the absorption spectra the aliquots were centrifuged at 10000 rpm/15 min and the supernatant was passed through 100 nm pore size membranes to assure the absence of any trace of the solid sample.

Band gap Measurements: Band gap measurements were extracted from diffuse reflectance measurements carried out in a UV-vis spectrophotometer (Perkin Elmer Lambda 35) equipped with a P/N AA-00214-100 integrating sphere reflectance unit (Spectralon LABSPHERE).

*Photocatalytic Properties***:** The photocatalytic activities of the C80 samples heated at 550 ºC in N_2 were evaluated by the degradation of methylene blue (10^{-5} M aqueous solution) in a cylindrical quartz flask. The degradation was carried out under UV light (peak wavelength at 365 nm) from a 200 W Xe-Hg lamp. 25 mg of sample was dispersed into 100 mL of the dye solution. After stirring in the dark for 60 min the suspension was exposed to the UV light. UV-vis absorption spectra (680 nm, Perkin Elmer Lambda 35) were recorded at different intervals to monitor the reaction. Prior to recording the absorption spectra the aliquots were

centrifuged at 10000 rpm/15 min and the supernatant was passed through 100 nm pore size membranes to assure the absence of any trace of the solid sample.

Table S1. Summary of the experimental conditions used to prepare the samples for a volume of cyclohexane of 0.5 L. The surfactant concentration was set in all cases at 0.2 M. *C and D* stand for concentrated and diluted reactants concentration. 80 and 120 stand for the final temperature.

Sample	$TiOSO_4$ (1.25M) (mL)	H_2O (mL)	Anatase seeds (0.2 M) (mL)	Final Temperature (ºC)	time remaining at 60 ºC (h)
C80	25	20	5	80	5
C120	25	20	5	120	5
D80	12.5	16	2.5	80	10
D120	12.5	16	2.5	120	10

Figure S1: Pore size distribution of sample C80 heated at a temperature where all the surfactant has been eliminated and at a lower temperature where the residual surfactant still remains in the sample. The reduction in pore size for the sample heated at lower temperature clearly indicates that interstitial surfactant is present on the samples. Anatase crystallite size in both samples was similar, which indicates that no sintering process has taken place and that pore coalescence has not taken place either when increasing temperature. Thus, the results can be unequivocally associated with the presence of interstitial surfactant.

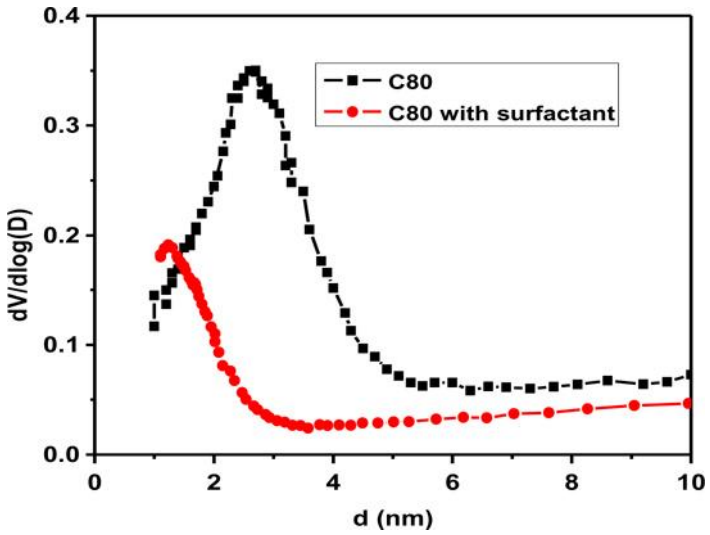

Figure S2: TEM pictures of the samples (magnification is the same to the one shown for C80). Size distribution histograms and mean and standard deviation data are also shown.