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Supplementary Information

Suppression of the Hemolytic Effect of Mesoporous Silica Nanoparticles after Protein Corona Interaction: Independence of the Surface Microchemical Environment

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Experimental

Materials

Tetraethyl orthosilicate (TEOS) was purchased from Acros Organics, Belgium. Ammonia solution (28%-volume), hydrochloric acid (HCl), absolute ethanol (CH_3CH_2OH) and buffer solutions (pH = 4, 7 and 10) were purchased from LabSynth, Brazil. Cetyltrimethylammonium bromide (CTAB), (3-aminopropyl)triethoxysilane (APTES), aqueous solution of 3-(trihydroxysilyl)propyl methylphosphonate (THSPMP, 42%-weight) and PBS tablets were purchased from Sigma-Aldrich, USA. All reactants previously mentioned had at least analytical grade and were used as received. Deionized water was produced in a Milli-Q system (Millipore, USA).

CTAB extraction from products

After isolated, all products were submitted to a extraction process in order to remove the soft template (CTAB), which is stabilized in the pores due to electrostatic interactions between its hydrophilic head ($–N(CH_3)_3^+$) and surface deprotonated silanols ($SiO^–$). For the elimination of CTAB, samples were redispersed in a concentration of approximately 10 mg mL^{-1} in a mixture of absolute ethanol and hydrochloric acid (9:1 volume ratio), and sonicated for 10 min. The efficiency of the extraction process is confirmed by thermogravimetric analysis (see Figure S4), which indicated the significant reduction of the weight loss up to about 300 °C, related to the decomposition of CTAB.

Table S1. Quantity of TEOS and organosilanes for surface functionalization

Sample	Co-condensation		Post-grafting
	t = 0 min (A)	t = 90 min (B)	(C)
Si–OH[a]	TEOS = 2.50 mL (100% mol of Si)	-	-
$Si–P(CH_3)O_3H$[a]	TEOS = 2.50 mL (100% mol of Si)	THSPMP = 127.7 µL (+2.5% mol of Si)	-
$Si–NH_2$[b]	-	-	APTES = 730 µL (+25% mol of Si)

[a]The following reactants had the same quantity used for all syntheses: CTAB = 0.75 g; absolute ethanol = 3.20 mL; NH_3 solution (0.048 mol L^{-1}) = 20.0 mL. The syntheses were performed at 60 °C for 120 min. [b]This sample was obtained starting from sample Si–OH. The calculation of the % mol of Si is relative to the weight of sample Si–OH used for the post-grafting process (290 mg).

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Characterization

Thermogravimetric analyses (TGA) were done in a thermal analyzer (TA Instruments 500, TGA module 2050) with the analyses performed by using approximately 3.0 mg of dried samples placed in an alumina crucible. The heating rate was maintained at 5.0 °C min^{-1} and air flow rate at 100 mL min^{-1}. Nuclear magnetic resonance spectra (NMR) of ^{13}C were acquired in a Bruker Avance 300 MHz, by using the CPMAS method (cross polarization and magic angle spinning of neighboring ^{1}H nuclei) with 20480 scans, acquisition time of 0.0499 s, pulse interval of 3.0 s and frequencies of 75.475 MHz in channel 1 (^{13}C) and 300.131 MHz in channel 2 (^{1}H). The analysis is done with an excitation pulse (90°) of 2.7 μs in channel 2, followed by a pulse of 4000 μs in channel 1, followed by the signal acquisition. Along the generation of the pulse in channel 1 (^{13}C), another pulse is generated in channel 2 (^{1}H) for decoupling, which is kept until the relaxation of the channel 1 signal. The chemical shift (δ, in ppm) is related to the molecule $CHCl_3$. ^{29}Si NMR spectra were acquired in a Bruker Avance 300 MHz through the HPDEC method (high-power decoupling, $^{29}Si \rightarrow {}^{1}H$), with 1024 scans, acquisition time of 0.0299 s, pulse interval of 60.0 s and frequency of 59.624 in channel 1 (^{29}Si) and 300.130 MHz in channel 2 (^{1}H). The analysis was done with a unique pulse excitation (90°) of 5.0 μs in channel 1 (^{29}Si) followed by the acquisition signal along to a continuous decoupling pulse in channel 2 (^{1}H), initiated simultaneously with the channel 1 pulse. The chemical shift (δ, in ppm) is related to the molecule $Si(CH_3)_4$. The sample holder was kept under a constant spinning of 10 kHz for all experiments. Deconvolutions of ^{29}Si NMR peaks were done in the PeakFit software, by using Gaussian functions. The morphology of the nanoparticles was analyzed by transmission electron microscopy in bright field mode (TEM-BF, Zeiss Libra 120, operating at 80 kV). Dynamic light scattering (DLS) and zeta potential (ζ) measurements were obtained on a Malvern ZetaSizer-Nano instrument. The particle size was determined by using a concentration of 50 μg mL^{-1} of silica nanoparticles with deionized (DI) water as the dispersant. To measure the size of the protein coating on each sample produced in this study, porous silica nanoparticles were dispersed in blood plasma media (55%) and the suspensions were centrifuged and washed several times with a phosphate buffer solution (PBS) in order to desorb weakly bonded proteins. Then, nanoparticles were resuspended in DI water and evaluated in the Malvern ZetaSizer-Nano instrument in a concentration of 50 μg mL^{-1}. Zeta potential measurements were taken by mixing 250 μL of a suspension of nanoparticles in deionized water (1.0 mg mL^{-1}) with 250 μL of a buffer solution (pH 4, 7 and 10), and then adding more 3.0 mL of deionized water. The analysis is done with 1.0 mL of the final suspension. Nitrogen sorption experiments were carried out at liquid nitrogen temperatures on a ASAP 2020. The samples were treated at 120 ºC for 12 h prior to the measurements. The specific surface area was calculated from the N_2 adsorption branch using the BET method. The pore diameters and volumes were estimated from the N_2 adsorption branch using the BJH method and from the single-point value adsorbed at the relative pressure (P/P_0) of ca. 0.94, respectively.

Results

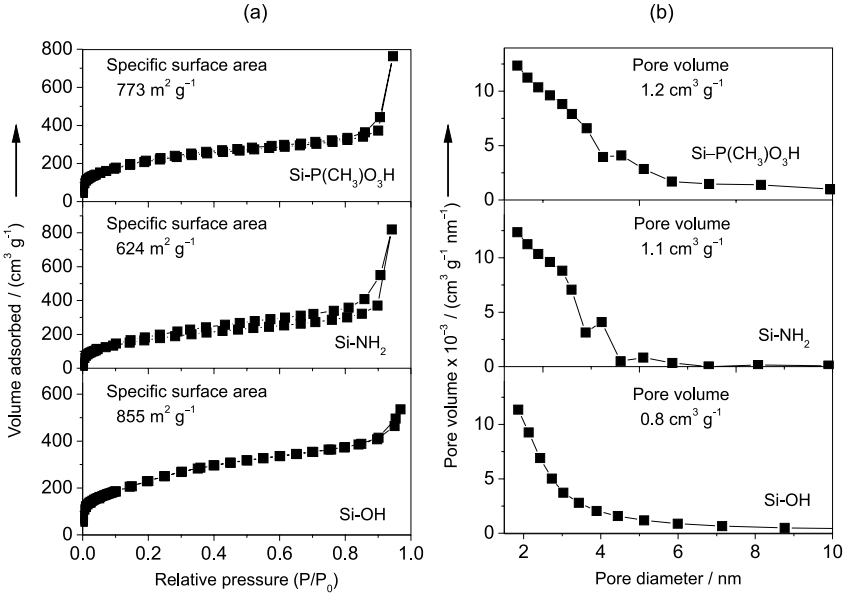

Figure S1. (a) N_2 adsorption-desorption isotherms and (b) pore size distributions of samples Si–OH, Si–NH_2 and Si–$P(CH_3)O_3H$.

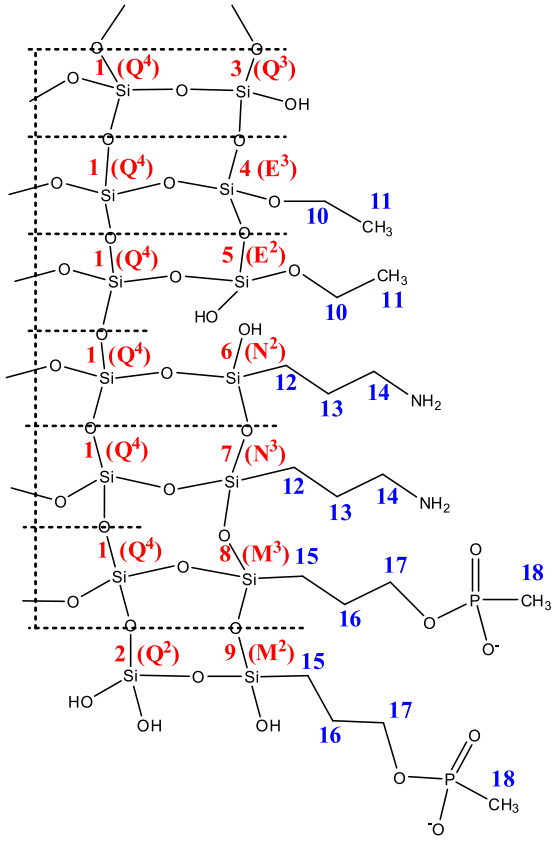

Figure S2. Nanoparticles chemical structure. The letters and numbers on silicon and carbon atoms are associated with the NMR peaks in the spectra shown bellow (Figure S3).

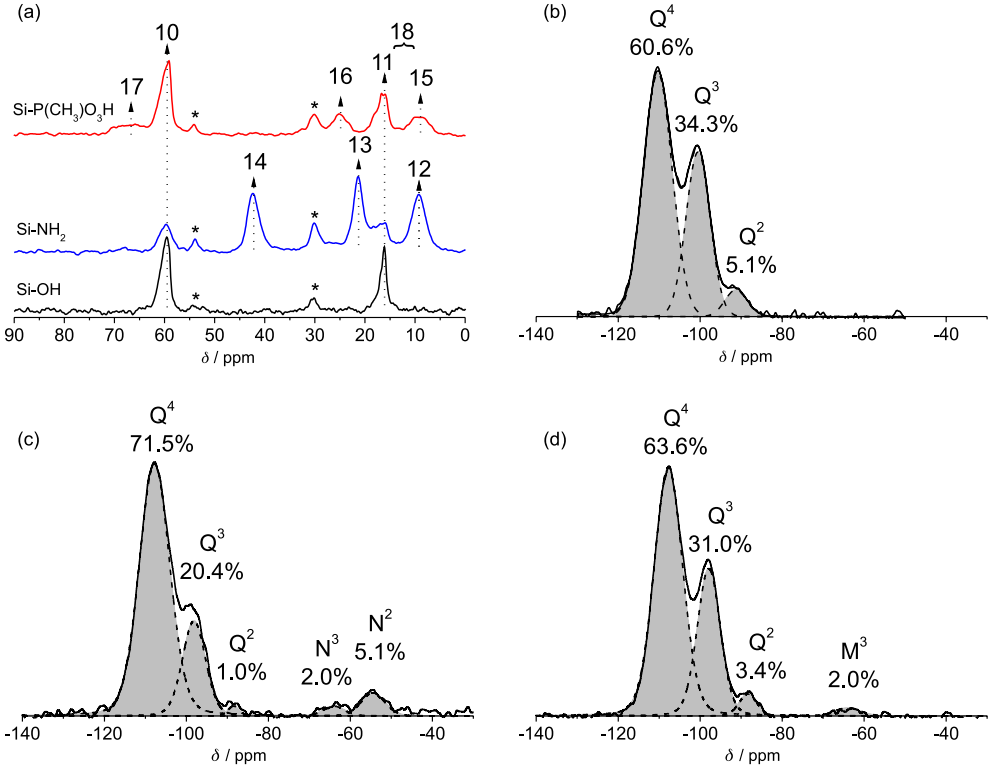

Figure S3. (a) ^{13}C NMR spectra of samples Si–OH, Si–NH_2 and Si–$P(CH_3)O_3H$. ^{29}Si NMR spectra of samples (b) Si–OH, (c) Si–NH_2 and (d) Si–$P(CH_3)O_3H$. *Peaks related to the alkyl chain of the residual CTAB after the extraction process.

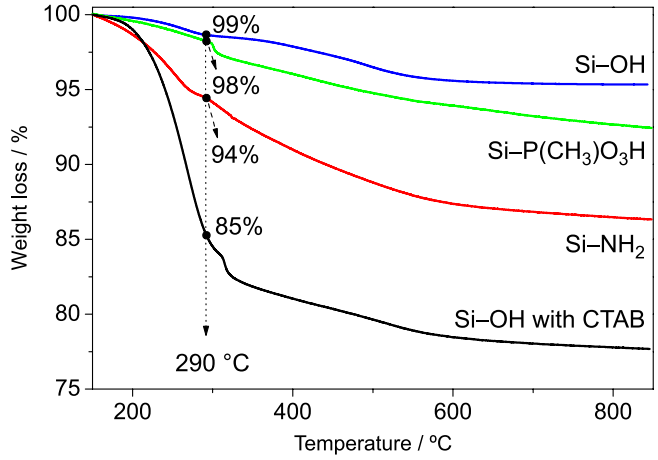

Figure S4. Thermogravimetric analyses of samples Si–OH, Si–NH_2 and Si–$P(CH_3)O_3H$.