

# Biocatalytic Elimination of Pharmaceuticals Found in Water with Hierarchical Silica Monoliths in Continuous Flow

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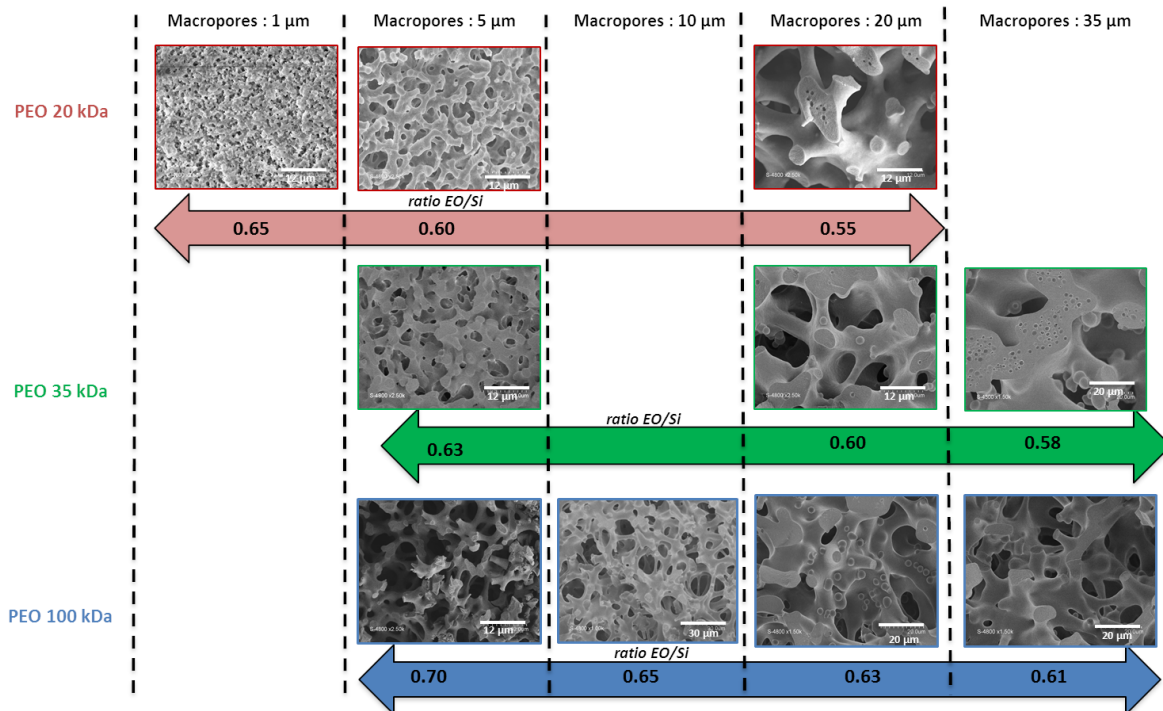
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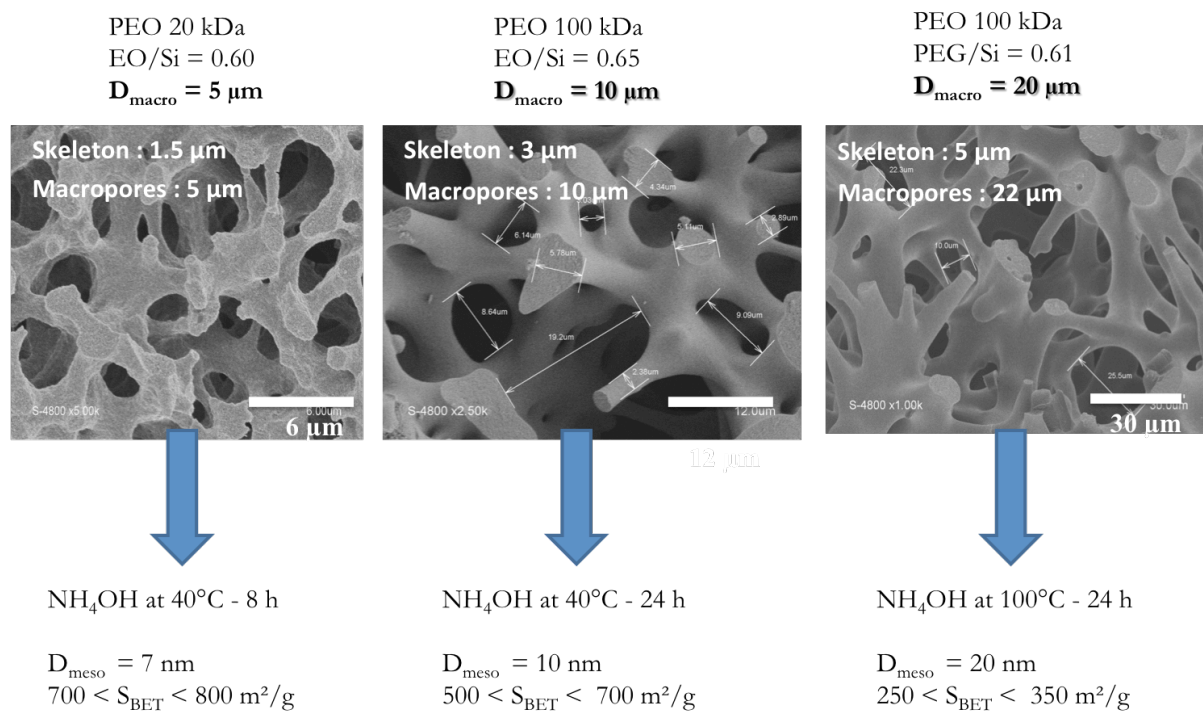
## Abstract

Pharmaceutical products (PPs) are considered as emerging micropollutants in wastewaters, river and seawaters and sediments. The biodegradation of PPs, such as ciprofloxacin, amoxicillin, sulfamethoxazol, tetracycline by enzymes in aqueous solution was investigated. Laccase from *trametes versicolor* was immobilized on silica monoliths with hierarchical meso-/macropores. Different methods of enzymes immobilization were experienced. The most efficient process was the enzyme covalent bonding through glutaraldehyde coupling on amino-grafted silica monoliths. Silica monoliths with different macropore and mesopore diameters were studied. The best support was the monolith featuring the largest macropores diameter (20  $\mu\text{m}$ ) leading to the highest permeability and the lowest pressure drop and the largest mesopore diameter (20 nm) insuring high enzyme accessibility. The optimized enzymatic reactor (150 mg) was used for the degradation of a PPs mixture (20 ppm each in 30 mL) in a continuous recycling configuration at a flow rate of 1 mL/min. The PPs elimination efficiency after 24 h was as high as 100% for amoxicillin, 60% for sulfamethoxazol, 55% for tetracycline and 30% for ciprofloxacin.

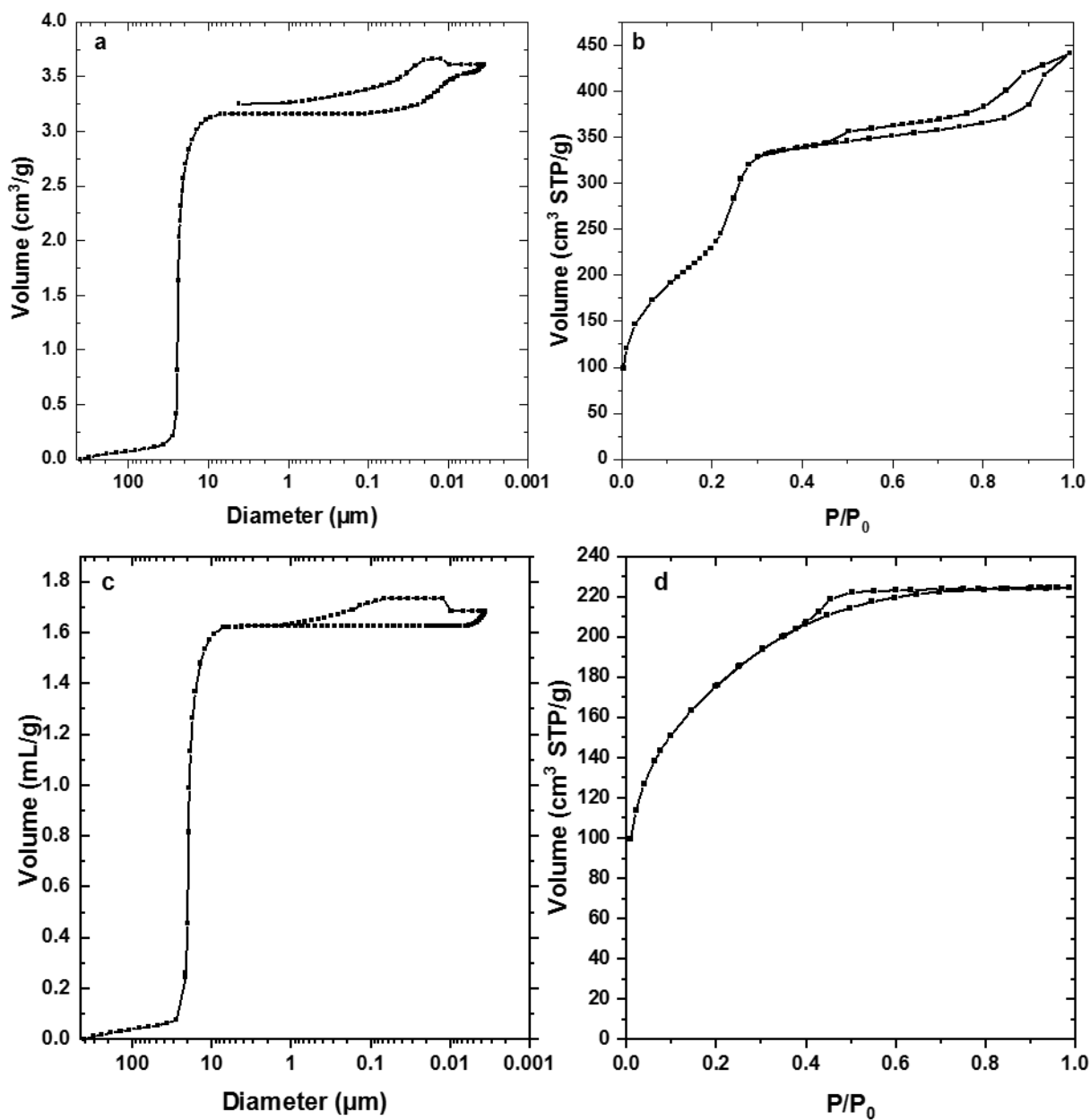
**Keywords:** silica monolith, laccase, water treatment, enzyme immobilization, pharmaceutical micropollutant, X-Ray tomography



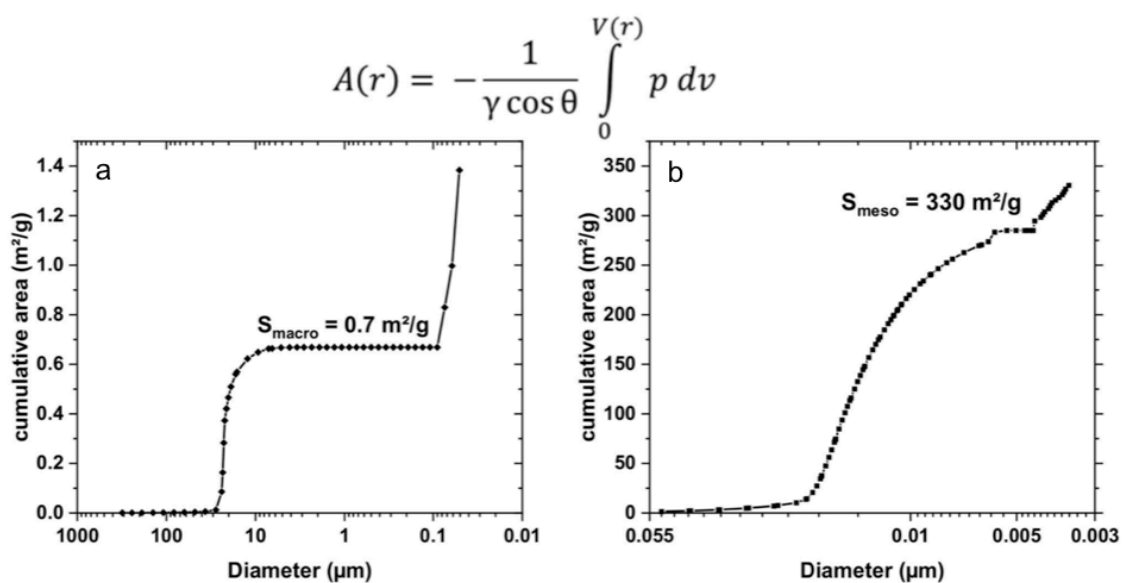
**Figure S1.** Control of macropore diameter in silica monoliths prepared by spinodal decomposition as a function of polyethylene oxide (PEO) molecular weight and of molar ratio of polymer (in EO unit) to silica.



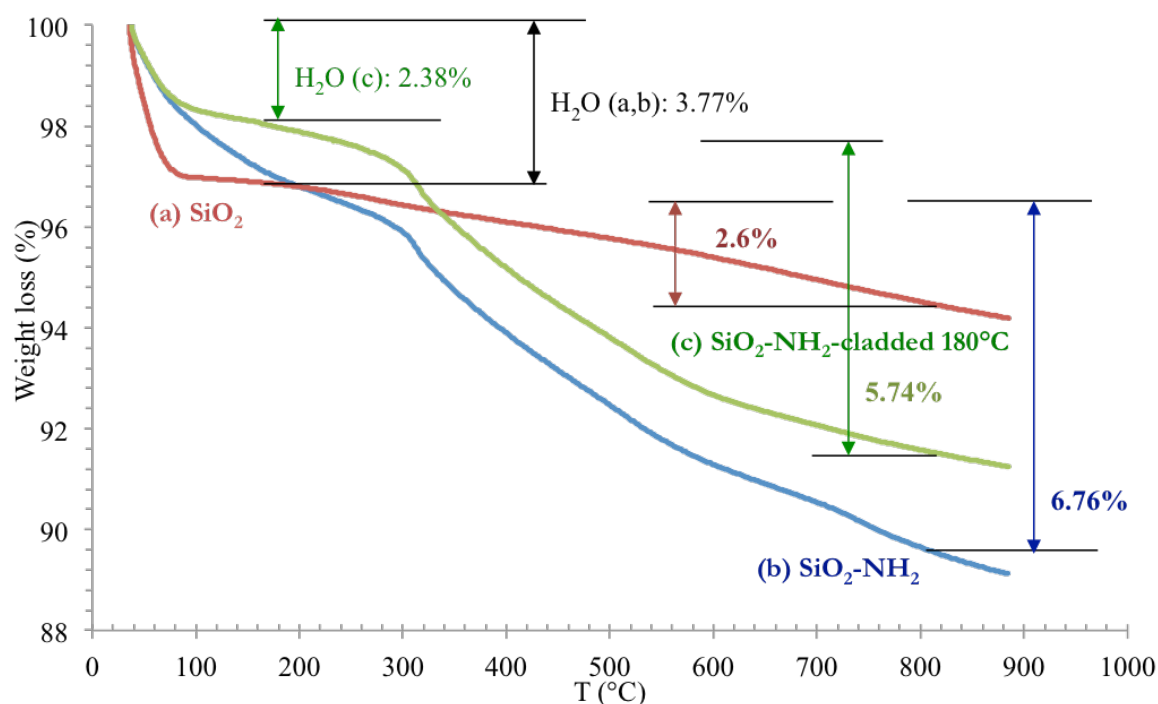
**Figure S2.** SEM images of Si-5-7, Si-10-10 and Si-20-20 and mesopore diameters and surface areas obtained after different basic ( $NH_4OH$ ) post-treatments.



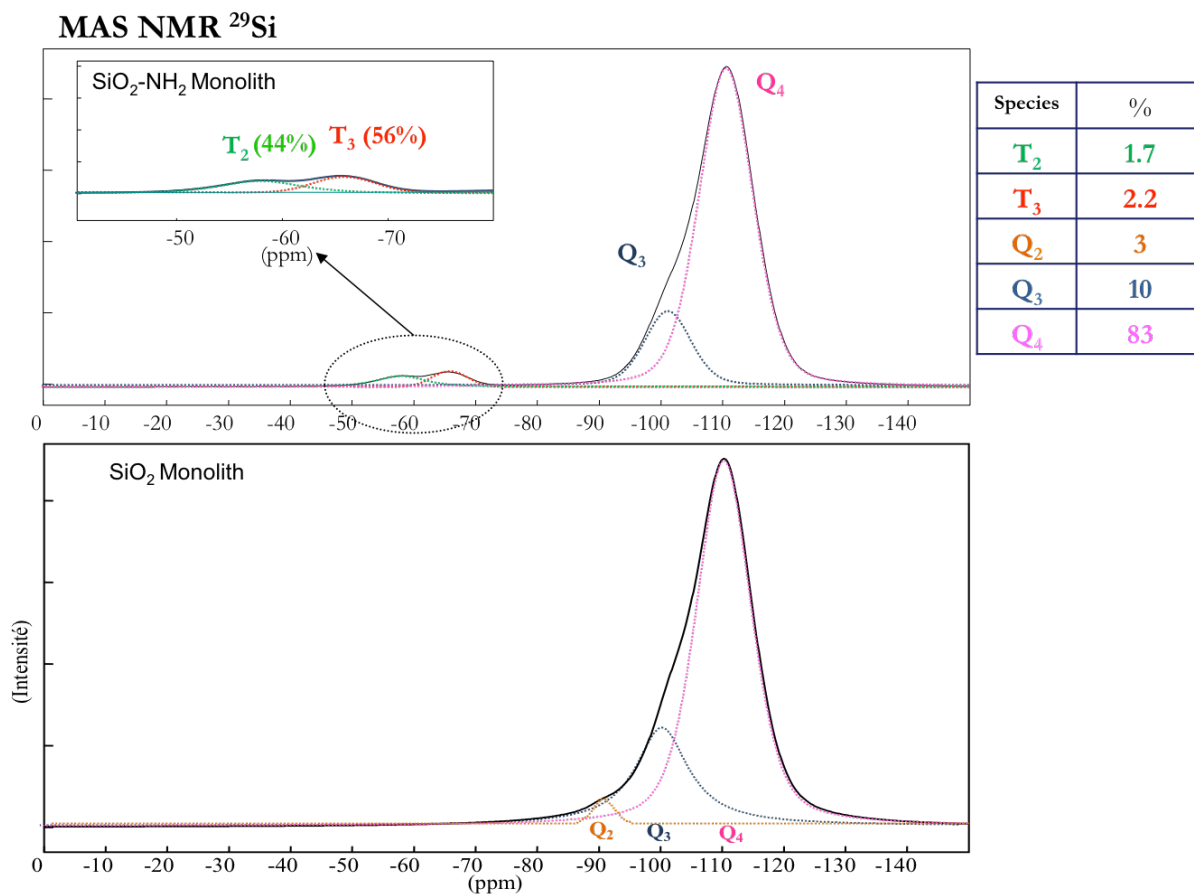
**Figure S3.** (a) Mercury porosimetry and (b) nitrogen sorption isotherm at 77 K of silica monoliths with 20 μm macropore diameter and (up) with a MCM-41 like mesoporosity (Si-20-(3,15) and (down) without basic post-treatment to avoid the formation of mesopores (Si-20-(<5)).



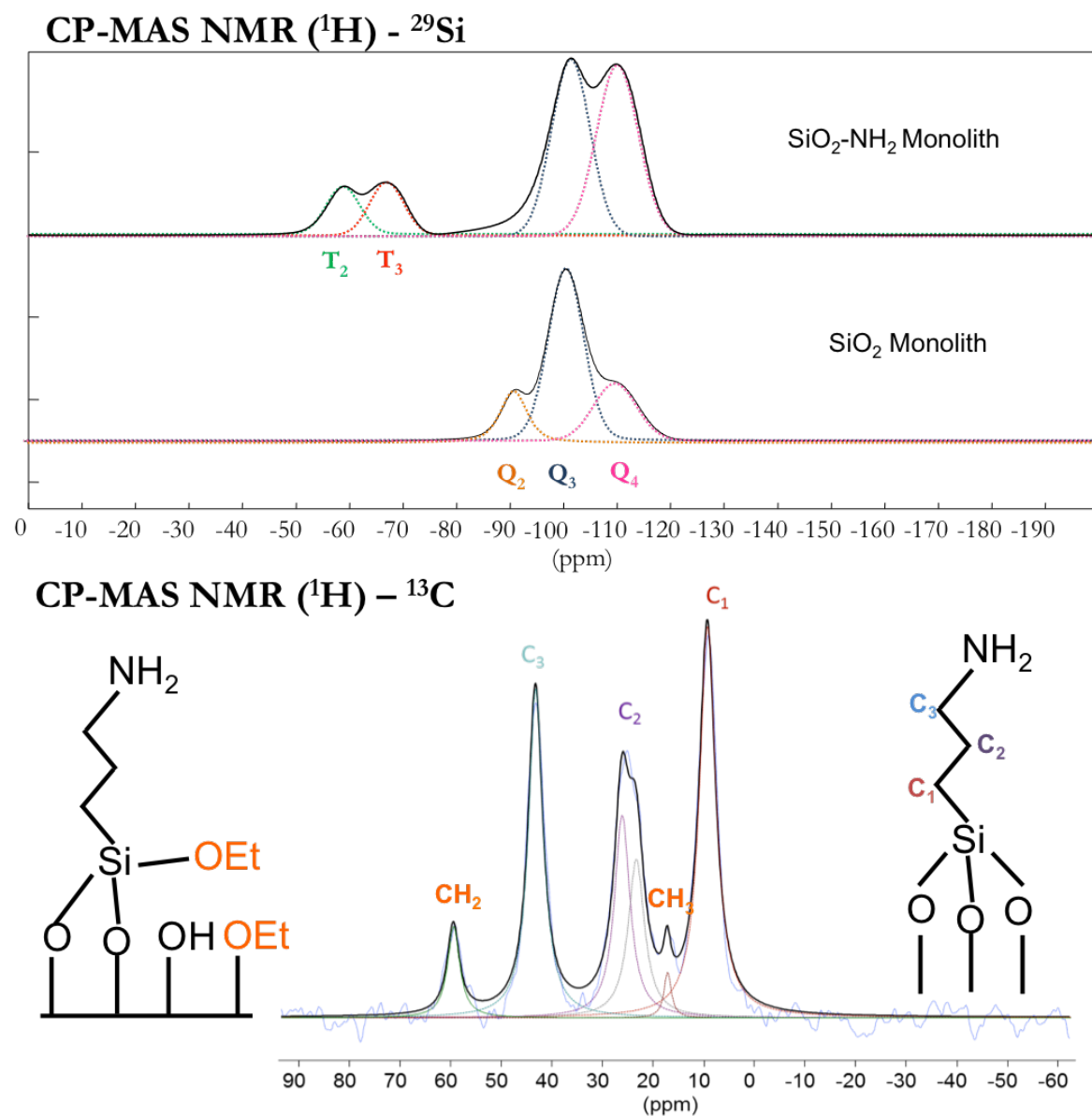
**Figure S4.** Calculation of specific surface areas from Hg porosimetry (intrusion branch) in the domain of (a) macropores and (b) of mesopores (Ex. Si-20-20)



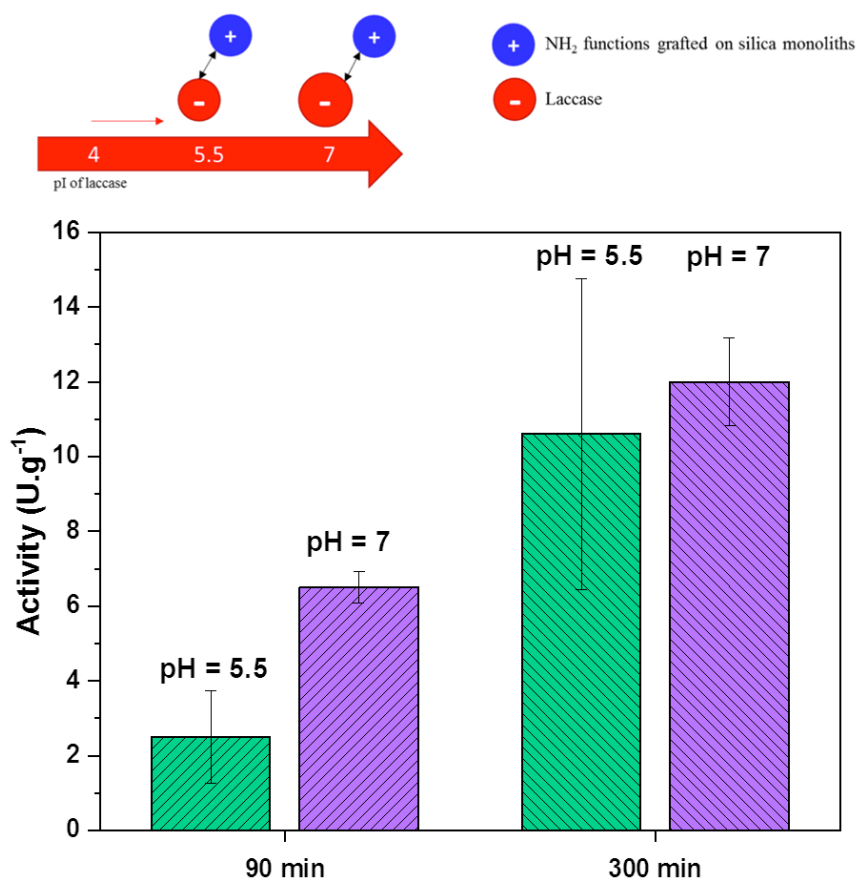
**Figure S5.** TGA of (a) Si-20-20, aminopropylsilane grafted Si-20-20 (b) before and (c) after the cladding at 180 °C.



**Figure S6.**  $^{29}\text{Si}$  MAS NMR of (down) Si-20-20 monolith and (up) aminopropylsilane grafted Si-20-20 monolith before cladding.

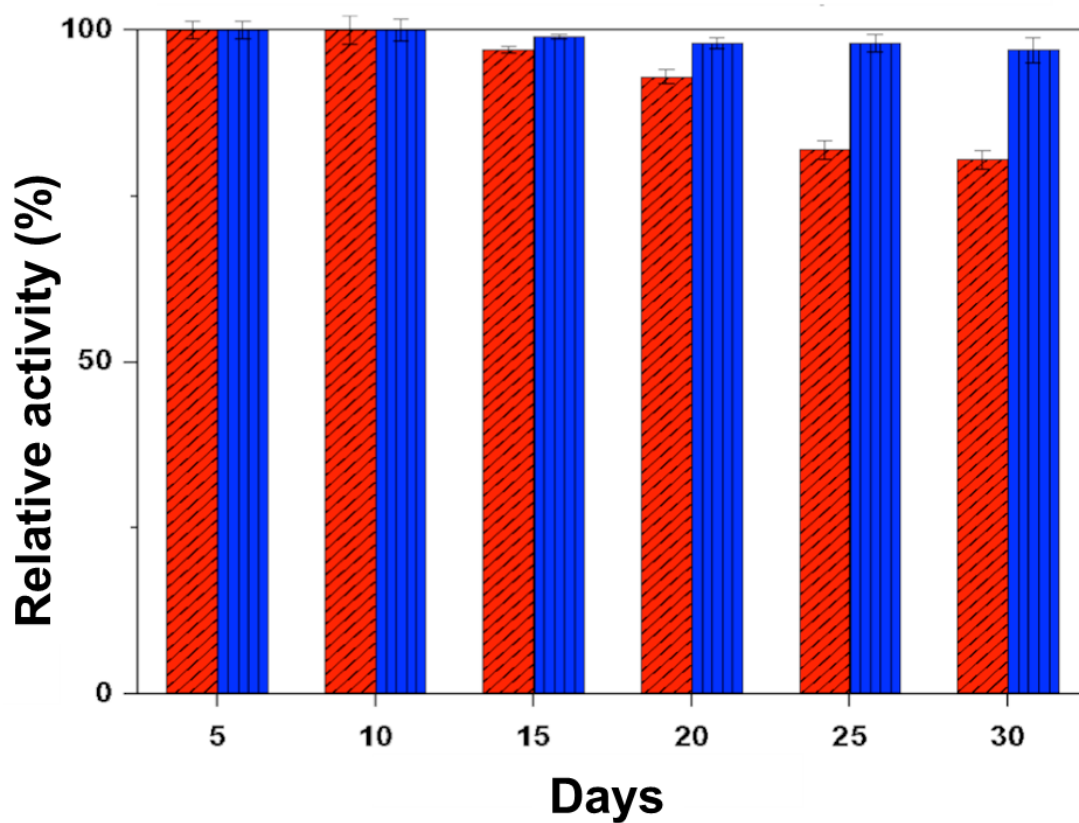


**Figure S7.** (up) ( $^1\text{H}$ ) $^{29}\text{Si}$  CP MAS NMR of Si-20-20 monolith and aminopropylsilane grafted Si-20-20 monolith and (down) ( $^1\text{H}$ ) $^{13}\text{C}$  CP MAS NMR of aminopropylsilane grafted Si-20-20 monolith before cladding.

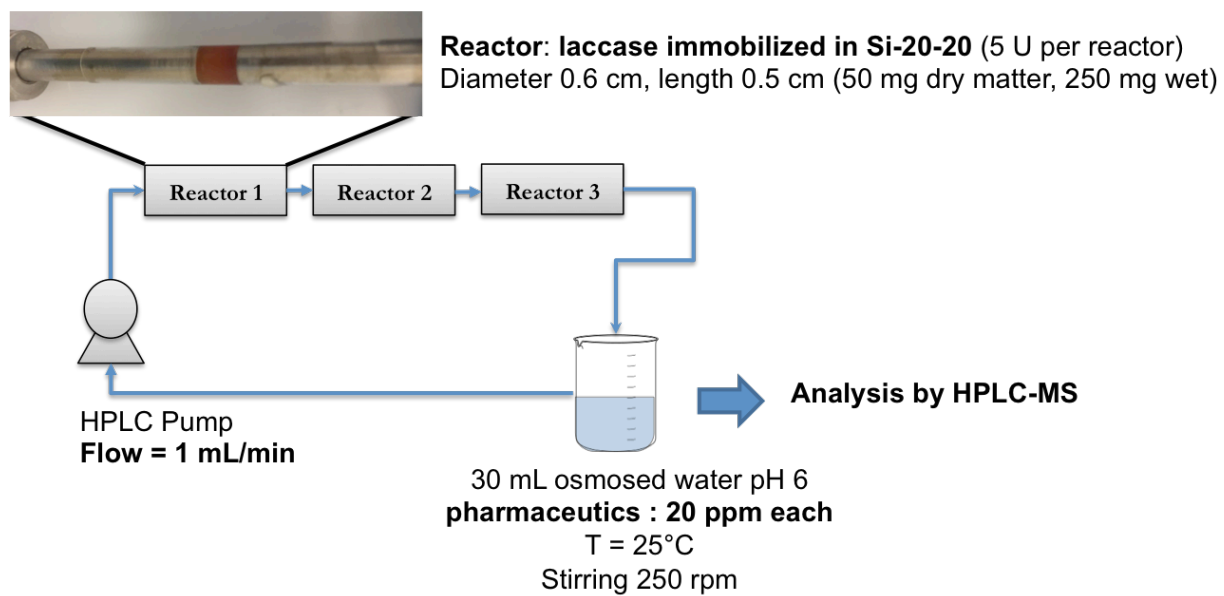


**Figure S8.** Schematic representation of the electrostatic interactions of laccase and the aminopropyl groups of the monoliths as a function of pH; and specific activity (ABTS test) of the laccases immobilized in aminopropyl-monoliths Si-20-20 at pH 5.5 and 7 after different reaction times of immobilization 90 and 300 min.

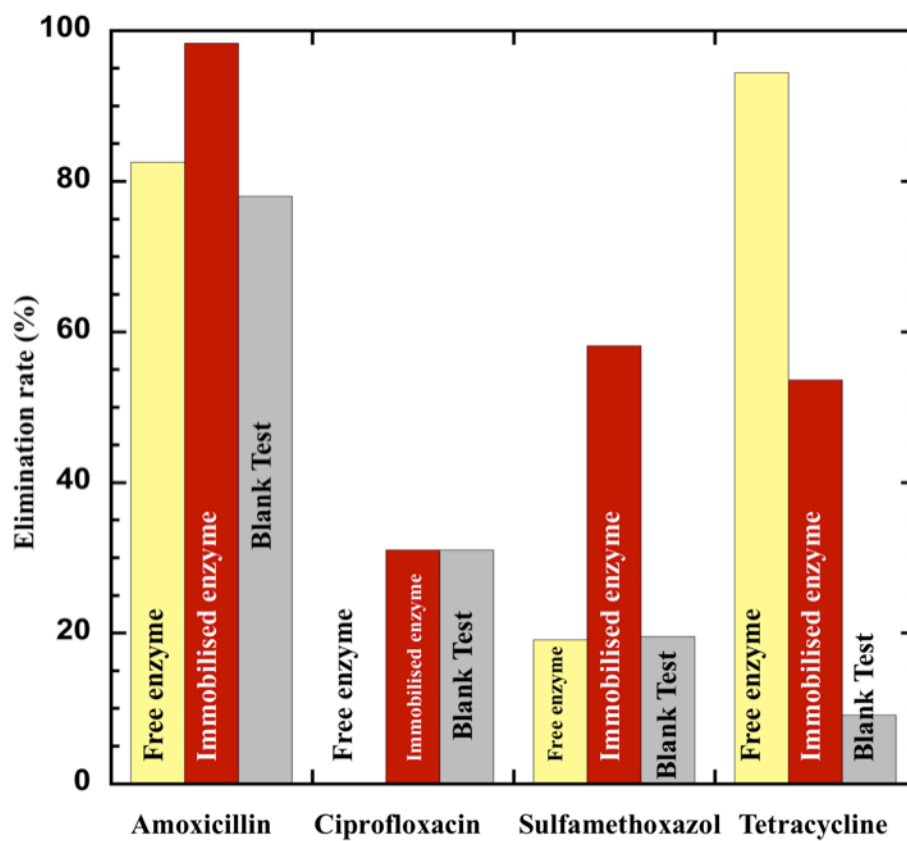




**Figure S9.** Specific activity (ABTS test) of laccases (red) immobilized on Si-20-20 by covalent coupling and (blue) of free laccases as a function of storage time in buffer solution at pH 7 at 4 °C.



**Figure S10.** Schematic representation of the bioreactors set up for water treatments.



**Figure S11.** Percentage of elimination of pharmaceutical molecules with (red) laccases immobilized in Si-20-20 by covalent binding, (yellow) free laccases in solution, (grey) blank test with deactivated laccases (at 100 °C) in monoliths Si-20-20.