



# New non-covalent functionalized phenyl-methyl-silica for biomolecules immobilization: Experimental and theoretical insights of interactions



Claudia Bernal<sup>a,\*</sup>, Frank Ramirez<sup>b</sup>, Juan C. Poveda-Jaramillo<sup>c</sup>, Monica Mesa<sup>b</sup>

<sup>a</sup> *Tecnología Enzimática para Bioprocesos, Departamento de Ingeniería de Alimentos, Universidad de La Serena, Raul Bitran, 1305, La Serena, Chile*

<sup>b</sup> *Grupo Ciencia de los Materiales, Instituto de Química, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia UdeA, Calle 70 No. 52–21, Medellín, Colombia*

<sup>c</sup> *Laboratorio de Resonancia Magnética Nuclear, Parque Tecnológico de Guatiguara, Universidad Industrial de Santander, Bucaramanga, Colombia*

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## ABSTRACT

A new strategy for functionalization of porous silica with phenyl-methyl groups is developed and demonstrated in this work. Bare silica, a hierarchical bimodal porous material, was functionalized with non-hydrolysable alkylsilane (trimethylphenylsilane) by liquid phase grafting, without the use of additional catalyst. The analyses carried out by FTIR, zeta potential and <sup>29</sup>Si NMR of modified material indicating that the silane is efficiently and stably adsorbed on silica surface. Computational simulations showing the interaction between silane and silica is by formation of bridges between phenyl ring of silane and silanol groups of silica, with interaction energies similar to those found for hydrogen bridges. Additionally, this modification generates a change on hydrophobicity of silica (11% more hydrophobic than bare silica) that allows the success immobilization of lipase from *Thermomyces lanuginosus*, used as probe biomolecule. This biocatalyst exhibits a specific activity of 45.7 IU (50% more than those obtained with bare silica) and a half-life of 89.9 h at 45 °C in acetone, which is 5-fold higher than for the biocatalyst prepared with the bare silica. These results could contribute to the preparation of new and efficient materials to be used as supports for biocatalysts, delivery drugs and remediation.

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## 1. Introduction

The functionalization of porous silica has become of uppermost importance since it is considered an essential strategy for the development and design of devices for biotechnological, biomedical and environmental applications. It also offers many possibilities of tuning the physical and chemical properties of siliceous materials used as biomolecules carriers. The organic functionalization of porous silica permits the tailoring of the surface properties (hydrophilicity, hydrophobicity, charge and interactions with guest molecules); changing its surface reactivity and altering of the bulk properties of the material [1].

There are two ways to modify the chemical surface of porous silica: during the generation of the material (*one-pot synthesis*) or

postsynthesis (*grafting methodology*). The one-pot synthesis involves the co-condensation of alkoxy silane (typically tetraethoxysilane or tetraethoxysilane) with an organoalkoxy silane or bis-silylated precursor in solution. The later containing the desired functional group in its structure. The resultant material is an organosilica, in which the functional groups are in the walls or in the surface, depending of the used organoalkoxy silane. The main disadvantage of this methodology is the effect of organoalkoxy silane on the pore structure and morphology of material [2]. The grafting methodology modifies the silica surface after the formation of material, using the silanol groups of the silica surface as point of reaction with organoalkoxy silanes or chlorosilanes [3]. The main advantage of this methodology is the selective modification of porous silica surface (external surface, pore surface, pore entrances) without affecting the porous structure [2].

Generally, the grafting is carried out by covalent attachment of an alkoxy silane, which is hydrolyzed for its activation and after that, the nucleophilic attack to a silicon atom of the siloxane bridge occurs [4]. The post-synthesis functionalization has been also used

\* Corresponding author.

E-mail addresses: [clabertz@gmail.com](mailto:clabertz@gmail.com), [cbernal@userena.cl](mailto:cbernal@userena.cl) (C. Bernal), [mmesacad@gmail.com](mailto:mmesacad@gmail.com), [monica.mesa@udea.edu.co](mailto:monica.mesa@udea.edu.co) (M. Mesa).

for the substitution of silica with metalorganic compounds (Gignard chemistry) [2]. This strategy can be carried out by vapor or liquid phase. In the later, the presence of water or organic solvent affects the location of the organic group (entrance or surface pore) and the quantity of attached organic groups per gram of material [5,6]. The post-synthesis functionalization by vapor phase can be made by using alkylsilanes, but it is necessary the use of fluorinated or chlorinated reagents that active the reaction site on the silica surface [7]. More recently, some reports have shown non-covalent functionalization of silica materials, but all of them involve a first functionalization layer, achieved by chemical condensation, that allow the posterior functionalization process through electrostatic interactions [8–10]. On our best knowledge, direct functionalization with alkylsilanes by non-covalent bonding has not reported yet.

The hydrophilic-hydrophobic balance of the silica chemical surface is fundamental for many applications such as enzyme immobilization [11–14], drug delivery [15], separation-purification process [16] and metal catalysis [17], due to wettability of surface and diffusion of substrates and products onto and out of the particles. The phenyl groups are an example of the hydrophobic groups that can be on the silica surface after a functionalization process. Beside of their hydrophobic character, they exhibit the additional possibility of  $\pi$ - $\pi$  interactions through its aromatic ring, which is advantageous for several applications [18]. Especially for enzyme immobilization, hydrophobic interactions have been broadly explored for lipases, due to their mechanism of interfacial activation, [19]. Only organosilicas prepared by one-pot methodology have been used for immobilization of formaldehyde dehydrogenase [20] and lipase from *Pseudomonas fluorescens* [21], showing the successful interactions of the phenyl groups with the enzymes.

We report the stable and non-covalent functionalization of porous silica with phenyl groups using a non-hydrolysable alkylsilane (trimethylphenylsilane) by liquid phase grafting. The NMR studies and computational simulation shows that the main interactions between silica network and alkylsilane are non-between siloxanes and hydrogens of aromatic ring, with energies similar to typical hydrogen bond. The chemical surface of this new material was broadly characterized here and it was applied as support for immobilization of lipase from *Thermomyces lanuginosus*, showing excellent results as enzyme carrier.

## 2. Experimental section

### 2.1. Chemicals

The following analytical grade reagents were purchased from Merck (Darmstadt, Germany) and used without further modification: ethylacetate (EtAc), sodium silicate (25–29% SiO<sub>2</sub> and 7.5–9.5% Na<sub>2</sub>O), cetyltrimethylammonium bromide (CTAB), trimethylphenylsilane (PMS), p-nitrophenyl butyrate (pNPB), Rose bengal dye, acetone, toluene, Lipase from *Thermomyces lanuginosus* (TLL) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

### 2.2. Synthesis and characterization of supports

#### 2.2.1. Silica synthesis

Synthesis of silica was carried out according to a previous work [14]. A typical synthesis procedure is describe below: A mixture with the following molar proportion of reagents: SiO<sub>2</sub>: Na<sub>2</sub>O: CTAB: EtAc: H<sub>2</sub>O = 1: 0.3: 0.24: 7.2: 193 was heated at 80 °C for 48 h under quiescent conditions. Then, the solid was calcined at 540 °C (heating rate: 1.5 °C min<sup>-1</sup>) for 3 h. This bare silica was identified with BS code.

#### 2.2.2. Non-covalent functionalization

In this procedure, 1 g of silica was activated under vacuum during 12 h, then was mixed with 30 mL of 5% trimethylphenylsilane in toluene solution and gently stirred under reflux for 5 h. After filtration, the solid was washed with acetone and abundant water, and finally dried at room temperature. This material was denominated as phenyl silica (PS).

#### 2.2.3. Silica characterization

The porous characteristics were determined from the Nitrogen adsorption isotherms of the silica samples, which were measured using the ASAP2020 instrument (Micromeritics) after activation at 100 °C for 12 h. The specific surface area was evaluated from the nitrogen adsorption isotherms using the Brunauer–Emmett–Teller (BET) method, and the pore size and mesopore volume were calculated using the Barrett–Joyner–Halenda (BJH) model.

The morphology of each material was evaluated by Scanning Electron Microscopy (SEM). The images were taken with a Jeol JSH 5500 LV microscope (JEOL Ltd. Tokyo, Japan) in a high vacuum mode, at the accelerated voltage of 10 kV. Samples were coated with a fine gold layer (about 20  $\mu$ m thick), using the ion coating JEOL JFC 1200 apparatus (JEOL Ltd. Tokyo, Japan). Distribution of carbon on silica material was investigated by C- and Si-mapping of the surface of the samples by SEM/EDS (Energy Dispersive Spectroscopy in the JEOL JFC1200 apparatus).

Thermogravimetric analyses (TGA) were carried out on TGA Q500 Instrument (TA instruments) for evaluating the organic content after the functionalization process. The samples were heated at 10 °C/min, from room temperature up to 600 °C, under nitrogen atmosphere. The chemical surface of the materials was characterized by different techniques. The Fourier Transformed Infrared (FTIR) spectroscopy (Spectrum One, Perkin Elmer) was used for determining the changes on the surface chemistry of porous silica particles before and after the functionalization process. Samples, previously dried at 30 °C under vacuum, were pellet with KBr and the FTIR was taken in transmittance mode. The spectra were obtained at room temperature with 32 scans and 4 cm<sup>-1</sup> of resolution. The Nuclear Magnetic Resonance (NMR) spectra were acquired using a Bruker Avance III instrument at 9.4T, operating at 79.47 and 400.05 MHz for the <sup>29</sup>Si and <sup>1</sup>H channels, respectively. Quantitative <sup>29</sup>Si spectra were acquired using an One-Pulse sequence with a  $\pi/2$  pulse at power level of 140 Watts and 5.2  $\mu$ s. Acquisition time was 20 ms and 1256 points, FID resolution of 50.18 Hz, spectral width 396.5 ppm, recycle delay 8.0s, 8 k scans were added to obtain the spectra. For the acquisition of <sup>29</sup>Si-<sup>1</sup>H CP-MAS spectra, the Harman–Hann conditions were reached using a radio frequency field of 100 KHz, contact time of 7 ms and <sup>29</sup>Si and <sup>1</sup>H power levels of 150 and 100 during the contact; a composite decoupling pulse program SPINAL64 was used with pulse length in decoupling program of 8  $\mu$ s and recycle delay of 1 s; finally, 65 k scans were added to obtain the spectra. The samples were rotated at 10 KHz under MAS conditions in the two set of experiments. Spectra scale were adjusted using Q8M8 (Sigma–Aldrich) as external reference. The <sup>13</sup>C CP-MAS NMR spectra were processed: Fourier transform, phase correction, peak picking, and deconvolution, using the TOPSPIN 3.5 processing software from Bruker.

The quantification of silanol groups was made from <sup>29</sup>Si NMR one-pulse experiments and from the quantification of adsorbed pyridine by TGA/FTIR analysis, following the procedure described by Ramirez et al. [22].

The surface charge was measured by Zeta potential (Horiba Zeta potential instrument) at 25 °C for samples dispersed in water at different pHs (in NaOH and HCl solutions). The surface hydrophobicity was evaluated by adsorption of Rose Bengal dye [23]. For

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