Protein-Templated Biomimetic Silica Nanoparticles

Erienne Jackson, ‡ Mariana Ferrari, ‡ Carlos Cuestas-Ayllon, ‡ Rodrigo Fernández-Pacheco, ‡ Javier Pérez-Carvajal, § Jesús M. de la Fuente, † Valeria Grazu, ‡ and Lorena Betancor*†‡

1Laboratorio de Biotecnología, Facultad de Ingeniería-Universidad ORT, 11100 Montevideo, Uruguay
2Instituto de Nanociencia de Aragón (INA), Universidad de Zaragoza, 50018 Zaragoza, Spain
3Instituto de Ciencia de Materiales de Madrid, CSIC, Sor Juana Inés de la Cruz 3, Cantoblanco 28049 Madrid, Spain
4Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC-Universidad de Zaragoza, c/Pedro Cerbuna 12, 50009 Zaragoza, Spain

Supporting Information

ABSTRACT: Biomimetic silica particles can be synthesized as a nanosized material within minutes in a process mimicked from living organisms such as diatoms and sponges. In this work, we have studied the effect of bovine serum albumin (BSA) as a template to direct the synthesis of silica nanoparticles (NPs) with the potential to associate proteins on its surface. Our approach enables the formation of spheres with different physicochemical properties. Particles using BSA as a protein template were smaller (∼250–380 nm) and were more monodisperse than those lacking the protein core (∼700–1000 nm) as seen by dynamic light scattering (DLS), scanning electron microscopy (SEM), and environmental scanning electron microscopy (ESEM) analysis. The absence of BSA during synthesis produced silica nanoparticles without any porosity that was detectable by nitrogen adsorption, whereas particles containing BSA developed porosity in the range of 4 to 5 nm which collapsed on the removal of BSA, thus producing smaller pores. These results were in accordance with the pore size calculated by high-resolution transmission electron microscopy (HTEM). The reproducibility of the BSA-templated nanoparticle properties was determined by analyzing four batches of independent synthesizing experiments that maintained their properties. The high positive superficial charge of the nanoparticles facilitated adsorption under mild conditions of a range of proteins from an E. coli extract and a commercial preparation of laccase from Trametes versicolor. All of the proteins were quantitatively desorbed. Experiments conducted showed the reusability of the particles as supports for the ionic adsorption of the biomolecules. The protein loading capacity of the BSA-based biomimetic particles was determined using laccase as 98.7 ± 6.6 mg·g⁻¹ of particles.

1. INTRODUCTION

Silica nanoparticles have attracted considerable attention from researchers due to their vast potential in biomedicine. Properties such as biocompatibility, low toxicity, and scalable availability have fuelled studies that span from applications in cell differentiation⁴ to drug or siRNA delivery systems⁵ and imagnetology.³ Moreover, the precise control of silica particle size, porosity, crystallinity, and shape strengthen their possibilities in nanobiotechnology.⁵ However, traditional methods for the synthesis of silica nanoparticles often require time-consuming processes, the use of organic solvents and/or surfactants, high temperatures, and other harsh sol–gel processing conditions.⁶ Efforts should be focused on the search for new synthetic routes for silica nanospheres involving lower costs and a lower environmental impact while simultaneously maintaining reproducibility and biocompatibility.

The use of biomimetic approaches in the production of inorganic nanostructures could be of great interest to the scientific and industrial community due to the relatively mild physical conditions needed for their synthesis.⁶ In particular, biomimetic silica can be synthesized as a nanostructured material with divergent morphologies within minutes under mild and green conditions.⁸ Inspired by silafins proteins used by unicellular diatoms,⁶ several studies have identified alternate aminated molecules as candidates for inducing silica precipitation from precursor compounds in vitro.⁸,¹¹,¹² Silafins also serve in vivo as structure-directing templates via self-assembling into larger aggregates.¹³ The influence of templates in silica biominalization has been studied in vitro using biological and nonbiological molecules. For example, bacterial flagella proved to be a promising biotemplate for developing the morphology-controlled synthesis of silica nanotubes,¹⁴ and chiral cationic gelators induced the formation of helical mesoporous silica nanotubes.¹⁴ These works demonstrate that the study of these templates in biomimetic silica formation not only augments its...
2. EXPERIMENTAL SECTION

Polyethylenimine (PEI, MW 1300), Bradford reagent, a molecular weight marker (MW 30 000–200 000), protamine, laccase from 
Trametes versicolor (EC 1.10.3.2, activity of ≥10 units per mg protein), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diaminonitrosure (ABTS) were from Sigma-Aldrich (St. Louis, MO). Tetramethyl orthosilicate was from Merck (Whitehouse Station, NJ). Sodium phosphate dibasic and sodium acetate were from Biopack (Buenos Aires, Argentina). Sodium dodecyl sulfate (SDS) and Triton X-100 were from AppliChem (Darmstadt, Germany). EDTA disodium was from J. T. Baker (Mexico). BSA solution was from New England Biolabs (Ipswich, MA). Gel filtration PD10 columns were from GE Healthcare (Buckinghamshire, U.K.). All other chemicals were analytical-grade reagents.

2.1. Synthesis of Biomimetic Silica Particles. A stock solution of polyethylenimine (PEI, 10%) was prepared in deionized water. Silicic acid was prepared by hydrolyzing tetramethyl orthosilicate (TMOS, 1 M) in 1 mM hydrochloric acid. The precipitation mixture consisted of 10 mL of 0.1 M sodium phosphate dibasic buffer pH 8.0, 2.5 mL of hydrolyzed TMOS, and 2.5 mL of PEI 10%. The mixture was agitated for 5 min at room temperature. The resultant silica particles (Si-PEI) were pelleted by centrifugation for 10 min (4600g), washed twice with sodium phosphate dibasic buffer (0.025 M, pH 7.0), and sonicated for 5 min to improve monodispersity. For silica-PEI-BSA particles, the precipitation mixture consisted of 10 mL of sodium phosphate dibasic buffer (0.1 M, pH 8.0) with 1 mg mL⁻¹ BSA, 2.5 mL of hydrolyzed TMOS (1 M), and 2.5 mL of PEI 10%.

2.2. General Procedures for Nanoparticle Characterization. The morphology and particle-size distribution of the resulting nanoparticles (NPs) were characterized by SEM in a field-emission FEI Inspect F operated at 10 and 5 kV. Aliquots of the different lyophilized nanoparticles were immobilized on the SEM sample holder using double-sided carbon tape. The samples were then immediately sputter-coated with gold before observation. The particle-size distribution was evaluated from several micrographs using an automatic image analyzer. Approximately 100 particles were selected for further consideration, which resulted in stable size-distribution statistics. The nanoparticles were also analyzed under low atmospheric pressure and in the presence of water vapor using ESEM. Nanoparticles were added to a sample holder and mounted onto a Peltier cooling stages set at 3 °C. The relative humidity of the chamber was modified from 50 to 100%. Specimens were examined with a working distance of 5.5 mm and a low acceleration voltage of 10 kV at low pressure to reduce beam damage.
For HRTEM examinations, a single drop (10 μL) of the aqueous solution (0.1 mg·mL⁻¹) of BSA- and non-BSA-templated particles was placed onto a copper grid coated with a carbon film. The grid was air dried for several hours at room temperature. TEM analysis was carried out in an FEI Tecnai F30 working at 300 kV.

Thermogravimetric analysis (TGA) was performed using a TA STD 2960 simultaneous DTA-DTGA instrument in air or in a nitrogen atmosphere at a heating rate of 10 °C min⁻¹.

DLS and ζ-potential measurements were performed on a Brookhaven Zeta PALS instrument at 25 °C. Each sample was measured 3 times, combining 10 runs per measurement.

Textural properties of silica-PEI and silica-PEI-BSA were characterized by nitrogen adsorption isotherms at −196 °C of the samples using automatic adsorption equipment (ASAP2010 micromeritics). Before the measurements, samples were lyophilized and then calcined under air flux in a HOBERSAL oven at 600 °C for 6 h using a heating rate of 7 °C·min⁻¹. Lyophilized and calcined samples were then outgassed under vacuum at 150 °C until the pressure was stable and lower than 5 μmHg. The apparent BET surface area (S_BET) was calculated by fitting nitrogen adsorption data to the BET equation. The micropore volume (V_m) was calculated by the t-plot method. The Barrett–Joyner–Halenda (BJH) method was also applied in order to determine the pore size distribution in the mesopore range (2–50 nm). The total pore volume was directly recorded from the isotherm. The free space measurements were performed using helium gas. Nitrogen and helium gases used in the experiments were 99.9995% pure.

2.3. Determination of Protein Concentration. For E. coli experiments, the protein concentration was determined with the Bradford reagent using the protocol recommended by the manufacturer, using BSA as a standard. For laccase experiments, the protein concentration was determined spectrophotometrically by measuring the absorbance at 595 nm specific to T1 Cu located at the active site and using an ε_M = 4400 M⁻¹·cm⁻¹.

2.4. Determination of Laccase Enzyme Activity. The activity of Trametes versicolor (Tv) laccase was measured spectrophotometrically following the increment in the absorbance at 405 nm generated by the hydrolysis of ABTS (0.38 mM) in 25 mM sodium acetate buffer pH 4.5 at 25 °C. One enzyme unit (IU) was defined as the amount of enzyme able to produce 1 μmol of product per minute under the above-defined conditions.

2.5. Adsorption of Escherichia coli Total Protein Extract to Si-PEI-BSA Nanoparticles. E. coli BL21(DE3) cells harboring pUC18 were inoculated into Luria–Bertani (LB) medium supplemented with 100 μg·mL⁻¹ ampicillin and incubated overnight at 37 °C with continuous shaking (200 rpm) and then harvested by centrifugation at 5000 rpm for 15 min. Pellets were then resuspended in a lysis buffer (PBS, lysozyme 1 mg·mL⁻¹, EDTA disodium salt 0.1 mM). The lysate was kept on ice for 30 min, and then 0.2% Triton X-100 was added. This step was followed by sonication (130 W ultrasonic processor, VCX130, Sonics and Materials, Inc., Newtown, CT, USA). The lysate was then centrifuged at 17 000g for 60 min at 4 °C, and the soluble fraction was collected.

Table 1. Dynamic Light Scattering and Net Charge Analysis of Nanoparticles Synthesized with Different Templates

<table>
<thead>
<tr>
<th>Sample</th>
<th>DLS size (nm)</th>
<th>PDI*</th>
<th>Z potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>silica-PEI</td>
<td>798.7 ± 100.8</td>
<td>0.639 ± 0.2</td>
<td>38 ± 0.8</td>
</tr>
<tr>
<td>silica-PEI-BSA</td>
<td>374.4 ± 36.4</td>
<td>0.409 ± 0.1</td>
<td>32 ± 0.7</td>
</tr>
</tbody>
</table>

*Values given in number.

Figure 1. Scanning electron microscopy (SEM) of (a) silica particles with PEI as a template, (b) graphical representation of the size distribution, (c) SEM of silica particles with PEI and BSA as templates, and (d) graphical representation of the size distribution.
Silica (0.5 g) was then added to a 2.5 mL solution of 10 mM sodium phosphate buffer pH 7.0 and 2.5 mL of the lysate soluble fraction. The mixture was incubated for 60 min at room temperature. Aliquots of supernatant collected by centrifugation (5 min, 5000 g) were taken at intervals of 10, 20, 30, and 60 min, and thereafter the mixture was incubated overnight at 4 °C with mild agitation and the final aliquot of supernatant was obtained. The protein concentration was measured for each aliquot, and the particles were washed three times with 10 mM sodium phosphate buffer pH 7.0.

2.6. Adsorption of Tv Laccase to Silica Nanoparticles. Enzyme stock solutions were prepared by dissolving the lyophilized enzyme in 25 mM sodium phosphate buffer, pH 7.0. The stock solutions were purified by gel filtration using PD-10 columns in 10 mM sodium phosphate buffer pH 7.0. One gram of silica was added to a solution of 2.5 mL of 10 mM sodium phosphate buffer pH 7.0 and 2.5 mL of gel-filtrated laccase (10 mg·mL⁻¹). The mixture was incubated for 60 min at room temperature. Aliquots of the supernatant collected by centrifugation (5 min, 5000 g) were taken at intervals of 10, 20, 30, and 60 min. The mixture was incubated overnight at 4 °C with mild agitation, and the final aliquot was obtained. The protein concentration was measured for each aliquot, and the particles were washed three times with 10 mM sodium phosphate buffer pH 7.0. The adsorbed laccase was then washed three times with 10 mM sodium phosphate buffer pH 7.0. The enzyme activity and protein concentration were measured for each aliquot.

2.7. Desorption of Proteins Adsorbed to Silica Nanoparticles. For desorption, silica particles containing adsorbed proteins were incubated with 10 mM sodium phosphate buffer pH 7.0 with 25, 50, 75, 100, and 300 mM NaCl (w/v 1:10 for laccase experiments and 1:25 for E. coli extract experiments). For a NaCl concentration of 1 M, the particles desorbed with 300 mM were washed three times and resuspended in 10 mM sodium phosphate buffer pH 7.0, 1 M NaCl. In each case, aliquots of the supernatant collected by centrifugation (10 min, 4600 g) were taken, and the enzyme activity and protein concentration were determined.

The visualization of adsorbed and desorbed protein patterns was done by polyacrylamide gel electrophoresis (SDS PAGE, 10%) followed by staining with Coomassie Brilliant Blue R-250. Besides, in the case of the adsorbed proteins, 0.1 g of silica after adsorption was washed three times with 10 mM sodium phosphate buffer pH 7.0 and then incubated with sample electrophoretic buffer (R 1:3) for 5 min at 100 °C.

2.8. Determination of the Loading Capacity of the Nanoparticles. Si-PEI and Si-PEI-BSA nanoparticles (0.025 g) were added to a solution of 0.5 mL of 10 mM sodium phosphate buffer pH 7.0 with 11 mg·mL⁻¹ Tv laccase. Also, 0.025 g of MANAE agarose was added to a solution of 0.5 mL of 10 mM sodium phosphate buffer pH 7.0 with 11 mg·mL⁻¹ Tv laccase. These mixtures were then incubated overnight at 4 °C with mild agitation. Aliquots of the supernatant collected by centrifugation (5 min, 5000 g) were taken. Enzyme activity was measured for each aliquot. The results shown in this article correspond to the mean value of the four independent experiments.

3. RESULTS AND DISCUSSION

In nature, as the presence of a template during synthesis alters the properties of the biosilica materials, we explored the use of
of a proteic inert BSA template during silica formation in vitro. Our hypothesis included that silica precipitation was achieved using PEI as the required aminated catalyst for the synthesis. PEI is a branched polymer bearing primary and secondary amines that has previously demonstrated its potential for the formation of nanosized silica particles.19 Interestingly, the use of BSA as a template generated particles with half the hydrodynamic radius as the particles without BSA (Table 1). BSA has net negative charge (pI 4.7) at pH 8.0, and the synthesis was conducted under this condition. Thus, PEI might cover the surface of a BSA core due to ionic interaction and therefore become organized as a compact sphere that directs silica precipitation. The difference in size could be advantageous for alternative applications of the biomimetic particles.24,25 In particular, large superficial areas increase the loading capacity of surface-immobilized biomolecules (e.g., proteins, nucleic acids, drugs) which would be highly beneficial for biotechnological and biomedical applied purposes because it allows us to obtain more efficient biocatalysts and it enhances the therapeutic efficacy of drugs.

Both BSA- and non-BSA-templated particles showed good colloidal stability with ζ potentials of 32 ± 0.7 and 38 ± 0.8 mV, respectively, at a neutral pH. Recommended values of the ζ potential for a stable colloid suspension must be superior to ±30 mV.26 Values of the ζ potential were greater than 30 mV from pH 3 to pH 6 (Supporting Information Figure S1).

Analysis by SEM showed that in the presence of BSA, biomimetic silica formed as preferentially loose particles (Figure 1a) with a nanosized diameter concentrated within the range of ~280–300 nm (Figure 1b). Whereas the absence of BSA during silica precipitation prompted the formation of interconnected randomly agglutinated particles (Figure 1c) of approximately 700–1400 nm (Figure 1d), the range was not as concentrated as in the presence of BSA.

This result is in accordance with those obtained for DLS (Table 1) that showed much larger hydrodynamic diameters for Si-PEI than for Si-PEI-BSA particles from the mean value obtained from four different batches of synthesis. Polydispersity index (PI) analysis also shows a wider size distribution in the case of non-BSA-templated particles. All of these results demonstrated that nanoparticles containing BSA had a more homogeneous size distribution (Table 1). This data confirmed our hypothesis of an ordered arrangement of PEI on the surface of BSA molecules, thus directing the formation of more compact particles.

High-resolution TEM analysis of silica particles provided evidence for spherical morphology nanoparticles with uniform nanopores (Figure 2). The nanopore size was estimated to be 2.6–3.4 nm for Si-PEI-BSA and 2.8–4.4 nm for Si-PEI. The morphology observed was spherical for Si-PEI-BSA nanoparticles with a confirmed distribution in sizes as shown by SEM and DLS (Figure 2b). In the case of Si-PEI, nanoparticles were found to be coalesced at times.
Both types of NPs were also analyzed by ESEM. The original characteristics of material samples analyzed by ESEM might be preserved because they do not need to be desiccated and coated with gold. Besides, it is possible to examine the samples in the presence of water vapor, thus obviating the need for a hard vacuum. ESEM micrographs taken at different percentages of relative humidity (from 50−100%) showed that the hydration process did not significantly alter the 3D morphology of both types of NPs despite their high content of organic matter (nearly 40% as determined by TGA analysis) (Figures 3 and 4). The size range of the nanoparticles was also maintained (Supporting Information Figures S2 and S3).

To characterize the textural properties of Si-PEI and Si-PEI-BSA nanoparticles, samples were lyophilized (lyo) and then calcined (cal). The nitrogen adsorption isotherm of Si-PEI-lyo presents a type II/III isotherm according to the deBoer classification which is characteristic of nonspecific adsorption (Figure 5). The absence of microporosity deduced from the t-plot method indicates that the organic compound (PEI) is located on the surface of the nanoparticles or inside the nanoparticles but blocks access to the sensing gas. Once the sample is calcined and the organic compounds removed (Si-PEI-cal), the isotherm can be classified as type II. S\text{BET} increases from 10 to 40 m\textsuperscript{2}/g, and the absence of microporosity is maintained (Table 2).

The difference between the knee of the isotherms suggests a higher affinity between the adsorbent and adsorbate on the calcined material. The organic compound was mainly located on the surface of the particles, and once PEI molecules were removed, the external surface area increased and thus a larger surface area of silica is now available for nitrogen adsorption. The apparent difference between these results and HTEM measurements could be explained by assuming that PEI molecules were also located inside the silica nanoparticles, blocking the access of the sensing gas in the lyophilized sample and thus enabling the measurement of mesoporosity by the BJH method. These pores could collapse during calcination, leading to nonporous silica nanoparticles or even porous nanoparticles with minuscule pores that are not detected by the limitation of the nitrogen adsorption technique. Lyophilized Si-PEI-BSA shows a type II isotherm with a mesopore distribution centered at 4.5 nm, which is in congruity with this material and with the pore size distribution calculated by HTEM. Nitrogen can flow inside these pores even though they still contain the

<table>
<thead>
<tr>
<th>Table 2. Textural Properties of Lyophilized (lyo) and Calcinated (cal) Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>sample</td>
</tr>
<tr>
<td>silica-PEI lyo</td>
</tr>
<tr>
<td>silica-PEI cal</td>
</tr>
<tr>
<td>silica-PEI-BSA lyo</td>
</tr>
<tr>
<td>silica-PEI-BSA cal</td>
</tr>
</tbody>
</table>

*a*Nondetectable.

Figure 4. ESEM micrographs of Si-PEI taken at different percentages of relative humidity: (a) 50, (b) 70, (c) 80, and (d) 100%. For clarification purposes, 50% humidity was equivalent to atmospheric moisture.
protein. Once the material is calcined, the external surface area decreases (Table 2) and the BJH method cannot be applied. Interestingly, a micropore volume (of less than 2 nm) (Table 2) can be determined. This can be explained by the collapse of mesopores when the proteins are denatured during the heating process, hence promoting smaller pores in the range of micropores.

After nanoparticle characterization, Si-PEI-BSA particles were assayed in the interaction with several proteins. Testing the nature of the interaction nanoparticles with biological samples is significant for their putative biotechnological application.

As an initial approach, nanoparticles were made to interact with a complex protein mixture. If ionic adsorption was the sole interaction between proteins with nanoparticles, then a complete desorption of the biological molecules would be expected after incubation with salt. The strength of ionic molecule adsorption on surfaces depends on the number of moieties involved in the interaction. Thus, poorly negatively charged molecules or small proteins that offer less surface area for interaction would desorb at low salt concentrations.

Experiments were conducted with different batches of silica synthesized particles offering a protein extract from *E. coli* to the particles. As the experiment was performed in batches, it was possible to analyze the differential adsorption of the 363 proteins from the supernatant by SDS-PAGE (Figure 6a). As expected, the electrophoretic pattern demonstrated that those 365 proteins with strong negative superficial charge were absorbed rapidly. There were no significant differences in the supernatants from 367 90 to 120 min (Figure 6a), and judging by the slope of the 368 curve showing protein content in the supernatant vs time, 369 proteins were absorbed mainly in the first 30 min of the 370 experiment (Figure 6b).

The adsorbed proteins were also analyzed by electrophoresis of the Si-PEI-BSA particles after boiling them directly in the
The colloidal stability of the particles adsorbed protein had been successfully desorbed (Supporting Information Figure S2). The particles as the pattern is significantly different from that of the initial protein extract (Figure 6a).

The stripped support was assayed again in the adsorption of proteins from E. coli total protein extract adsorbed to Si-PEI-BSA nanoparticles with different concentrations of NaCl. Lane 1: molecular weight marker. Lane 2: proteins adsorbed to silica before desorption. Lane 3: desorption of proteins with 25 mM NaCl. Lane 4: with 50 mM NaCl. Lane 5: with 100 mM NaCl. Lane 6: with 300 mM NaCl. Lane 7: with 1 M NaCl. (b) Curve representing the desorption of E. coli total protein extract adsorbed to silica nanoparticles with different concentrations of NaCl.

Desorption was quantitative, reaching 100.9 ± 5.4% protein recovery after incubation with 1 M NaCl (Figure 7b).

This experiment also proved that ionic interaction was the sole “communication” between proteins and the particles. Particles were completely devoid of adsorbed proteins after being incubated with NaCl (Supporting Information Figure S2). The stripped support was assayed again in the adsorption of proteins from E. coli extract, and similar results, in terms of protein adsorption and desorption, were obtained (Supporting Information Figure S4). Reusability constitutes a fundamental premise for ionic exchangers. Our results demonstrate the feasibility of reusing Si-PEI-BSA particles as ionic exchange material.

The same experiment as for the E. coli protein extract was carried out with a solution of a commercial well-characterized protein: a laccase from Trametes versicolor. Studies of the interaction of nanoparticles with a purified model protein may provide better insight into the charge specific binding and allow for rigorous studies of the maximum loading capacity. The laccase offered to the silica particles (0.4 mg·mL⁻¹) was adsorbed within the first 10 min of the experiment, as expected by its low pH of 4.5 and high negative net superficial charge. As laccases are often inhibited by Cl⁻, desorption was carried out by a shift to pH 3.0 and followed by measuring the increase in protein concentration in the supernatant. Again, quantitative amounts of enzyme were obtained, demonstrating that all of the adsorbed protein had been successfully desorbed (Supporting Information Figure S2).

4. CONCLUSIONS

The data summarized herein demonstrates that the use of an inert protein template (BSA) enabled the rapid synthesis of positively charged dispersed (250–380 nm) particles as opposed to the synthesis without a template which produced larger and smaller homogeneous nanoparticles. The characterization of different batches of nanoparticles demonstrated the reproducibility of the synthesis strategy. The particles synthesized in the presence of BSA reversibly and ionically adsorb a range of proteins. The nanoparticles proved successful in their reutilization, which added to their ease of preparation and cost-effective green synthesis which is a remarkable advantage of its use as a nanosupport for the surface integration of biomolecules. Although improved precise control of the 434 nanoparticle properties have yet to be developed, they present a promising perspective for their functionalization with proteins and their use thereof for biomedical, biosensing, or biocatalytic applications.

ASSOCIATED CONTENT

Supporting Information

Potential of Si-PEI-BSA and Si-PEI at varied pH and SDS-PAGE of the desorbed supports after incubation with NaCl, SDS, and silica reutilization. Size distribution of Si-PEI-BSA and Si-PEI nanoparticles at different percentages of relative humidity. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

E-mail: vgrazu@unizar.es. Tel: +34 976762982.
E-mail: betancor@ort.edu.uy. Tel: +59829021505.

DOI: 10.1021/la504978r
Langmuir XXX, XXX, XXX-XXX
ACKNOWLEDGMENTS

J.M.d.L.F. thanks ARAID for financial support. We also thank R. Barrios and T. García-Somolinos (Textural Characterization Facility at the Materials Science Institute of Madrid, Spain) for having carried out the N\textsubscript{2} adsorption/desorption experiments. E.J. and L.B. are grateful to the National Research and Innovation Agency (ANI1) and Universidad ORT Uruguay. J.P.-C. is grateful for MINECO (Spain) support (BES-2010-038410). We also thank Sara Rivera for technical support and Dr. C. R. I. Crick for his contributions to this article.

REFERENCES


