Optimization of the Properties of Macroporous Chromatography Silica Supports through Surface Roughness Control

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Silica supports could constitute interesting stationary phases for the separation of biological substrates by affinity chromatography because of their better mechanical strength compared to polymers. Pore sizes and surface areas of the supports should be large enough (>70 nm) to provide high binding capacity and high mass transfer. However, most commercially available large-pore silicas usually exhibit very low surface areas. In the present work, we show how to increase the surface area of macroporous (80 nm) low surface area (16 m^2/g) silica particles by a pseudomorphic transformation process occurring only at the surface of the pores, while preserving the macropores and the particle sizes of the parent silica. The transformation occurs through a controlled dissolution/reprecipitation mechanism in the presence of long-chain alkylammonium surfactants. Consequently, the surface area of these macroporous silica materials was increased by a factor of 10 to 20 and was tuned between 100 and 300 m^2/g. Moreover, this higher surface area allows the amount of functional groups immobilized at the surface of the materials to be increased by covalent grafting. The anchorage of glycidoxy groups at the surface of these new silicas produce materials with a high binding capacity for further protein coupling, comparable to polymeric phases. These new silica phases feature a high mechanical stability and represent an attractive alternative to polymeric phases in the area of large molecule separations.

Introduction

Affinity chromatography is a liquid chromatographic technique that uses a specific binding agent for the purification or analysis of sample components.[1] Interactions that occur in affinity chromatography are the same as those taking place in many biological systems, such as the binding of an enzyme with a substrate or of an antigen with an antibody. The very specific nature of these interactions leads to affinity chromatography with a high degree of selectivity. Cross-linked polysaccharides, agarose, sepharose, cellulose, or other synthetic polymers (polystyrene vinyl benzene, polymethacrylate) are widely used as support matrices. However, porous silica matrices with high mechanical strength can be alternatives for high throughput purification of antibodies. Diol-bounded silica was first reported in affinity chromatography in 1978[2] and provided a support with a hydrophilic and neutral coating. The latter constitutes a functional group that could be easily modified for ligand coupling and increases the stability of the silica support in elution buffers in the pH range from 2.5 up to 7.5.[3] Further improvements in the silica stability may be obtained by using a silica surface containing zirconium or alumina to work under more alkaline conditions.[4,5]

The combination of a high pressure liquid chromatography (HPLC) involving a silica support with an affinity ligand is now referred to as high performance affinity chromatography (HPAC). However, a limitation in using these supports for affinity chromatography concerns the pore size of the materials. For instance, pore sizes of 6−10 nm are commonly used in reverse phase HPLC (RP-HPLC), but these supports are not appropriate for many proteins. Silica with 70 to 400 nm pore sizes are available for HPAC, but these large pore materials generally feature low surface areas in the range 15−20 m^2/g.

Recently, Katoh et al.[6] have used a basic sodium hydroxide treatment to increase the pore size and the surface area of commercial silica having 70 nm pore size. Silica matrices with pore sizes from 70 to 180 nm and surface areas ranging from 26 to 67 m^2/g were obtained. These authors further showed that silica matrices having pore size larger than 100 nm showed four times higher mass transfer compared to cross-linked agarose (MabSelect) matrices.

In the present work, we have taken advantage of the experience gained from our previous studies on the prepara-

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Table 1. Textural and Surface Characteristics of the Parent FractoSil 800 and of the Surface Pseudomorphically Modified Materials FractoSil PM as a Function of Time of Transformation[a]

	N_2 adsorption			Hg porosimetry			
material	duration of treatment (h)	S_{BET} (m^2/g)	C_{BET}	D_{pore} (nm) (intraparticle)	V_{pore} (mL/g)	$D_{interpart}$ (μm)	$V_{interpart}$ (mL/g)
FractoSil 800	0	16.3	263	79	0.55	16	1.08
PM-93	3	93.6	306	93	0.83	21	1.35[b]
PM-178	4	178.1	63	98	0.84	27	1.65[b]
PM-235	6	235.4	92	100	0.78	22	1.59[b]
PM-456	20	456.6	78				

[a] The intraparticle mean macropore size is calculated in the 50−500 nm range. [b] Volume to bring particles closer has been removed from total volume (~0.65 mL/g) to evaluate interparticular volume.

tion of HPLC supports by pseudomorphic transformation of preshaped silica gels[7−11] to optimize the surface properties of macroporous silica particles for affinity chromatography. Previously,[7−11] we have shown how to transform the whole particles of amorphous mesoporous silica particles into ordered mesoporous silica of MCM-41 and MCM-48 type, without changing the particle size and the particle morphology of the initial silica. This process has also been successfully adapted to the transformation of silica skeleton of macroporous monolith into a MCM-41 type mesoporosity.[12] The procedure is based on the restructuration of the silica network of an amorphous silica body via a dissolution/reprecipitation process in basic media in the presence of surfactant micelles. The reaction is kinetically controlled and takes place inside each particle or skeleton. In the present work, this pseudomorphic transformation was adapted to amorphous macroporous (80 nm) silica, and by adjusting the kinetic parameters, we have achieved a pseudomorphic transformation restricted to the surface of the macropores leading to the creation of a surface roughness of around 10 nm wide and less than 10 nm depth without affecting the size of the macropore and the particle morphology and size. This opens the route to the use of macroporous silica in affinity chromatography with competitive properties toward polymeric phases used at the present time.

Experimental and Molecular Simulation Section

Materials. *FractoSil 800 and Modified FractoSils.* The parent material is a macroporous silica chromatographic support with a low surface area obtained as a gift from Merck (named FractoSil 800, Table 1). The pseudomorphic transformation of FractoSil 800 into MCM-48 type material was performed as described previously.[8] A solution of H_2O, NaOH, and cetyltrimethylammonium bromide (CTAB, Aldrich) is stirred at 55 °C to obtain a homogeneous solution. A quantity of 2 g of FractoSil 800 is then added under stirring, and the suspension is stirred for 2 h at 55 °C. Then, the mixture is put at 150 °C in an autoclave under static conditions for 5 h. The slurry is then filtered, washed with water, dried overnight at 80 °C, and calcined at 550 °C for 8 h. The mixture molar ratio was 1 SiO_2/0.38 NaOH/0.175 C_{16}TAB/120 H_2O. The stirring steps in the pseudomorphic synthesis were performed with an endless screw stirrer to preserve the morphology of the parent silica particles as described previously.[7−11]

The pseudomorphic surface modification of FractoSil 800 was performed as follows: 180 g of deionized water, 7.38 g of CTAB, 3.06 g of NaOH, and 0.72 g of NaI (SDS) were mixed at 55 °C during 15 min. A total of 5.32 g of decane (Aldrich) were added and stirred for 10 min. Then, 3.43 g of 1,3,5-trimethylbenzene (TMB, Aldrich) were added and stirred for another 15 min. A total of 15 g of FractoSil was then added to this solution and stirred at 55 °C for one more hour. The temperature was then raised at 90 °C, and the suspension was stirred for different times between 3 and 6 h to obtain modified FractoSils, named FractoSil PM and hereafter PM-X (X is the surface area which ranges from 80 up to 300 m^2/g). The reaction mixture corresponds to the molar ratio 1 SiO_2/0.075 C_{16}TAB/0.3 NaOH/0.02 NaI/0.11 TMB/0.15 decane/40 H_2O. The resulting solids were filtered, washed with water until neutral pH, and dried at 80 °C overnight. The materials are finally calcined at 550 °C under flowing air for 6 h.

Epoxy-Activated Silica. The technique of protein coupling often employed with silica begins with diol- or epoxy-activated silica.[13,14] We have previously shown that the more efficient activated groups to couple proteins on silica and more especially on micelle-templated silica (MTS) materials are glycidoxy groups.[15] This procedure has been therefore followed here. In a flask of 250 mL, 3 g of silica are left for 60 min at room temperature under argon flow to remove a part of the adsorbed water molecules. Then, 30 mL of anhydrous toluene are added as well as different amounts of glycidoxy propyltrimethoxysilane corresponding to 1.5 or 5 epoxy groups/nm^2 of dried silica. FractoSil PM obtained after 4 h and 6 h of pseudomorphic reaction have been functionalized with an initial mixture of 1.5 and 5 epoxy groups/nm^2, respectively, and Fractosil 800 with an initial mixture of 2.8 epoxy groups/nm^2. The mixture is heated under argon flow at reflux at 120 °C under magnetic stirring for 1 h and 30 min. The hydrolysis of the methoxy silane leads to the formation of methanol, which is distillated in the same amount of time with a Dean−Stark. The reaction is stirred for another 1 h and 30 min at 120 °C under argon flow. The resulting solid is filtered and washed with anhydrous toluene and then in a Soxhlet with a ether/dichloromethane mixture (1/1 in volume) at 60 °C overnight. The epoxy-activated silica is then oven-dried at 60 °C for 1 day.

Characterizations. The particle morphology was examined using a Hitachi S-4500 I scanning electron microscope (SEM). The

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Table 2. Properties of the Smooth (Surface A) and Rough (Surfaces B and C) Surfaces Considered in This Work

Silica Surface					
	surface (nm^2)	cavity diameter (nm)	cavity length (nm)	constriction diameter (nm)	cavity length (nm)
		Model A			
surface A	114.4				
		Model B			
surface $B_{1.0}$	133.2	6.0	1.0		
surface $B_{2.0}$	152.1	6.0	2.0		
surface $B_{3.0}$	170.9	6.0	3.0		
surface $B_{6.0}$	227.5	6.0	6.0		
		Model B			
surface $B_{1.0}$	212.4	6.0	1.0	2.0	2.0

particle size distribution was determined by laser diffraction (Mastersizer 2000 from Malvern Instrument): 200 mg of sample was added to 10 mL of acetone, ultrasonicated for 30 min, dried, and dispersed into 700 mL of water for analysis. Nitrogen sorption isotherms at 77 K were recorded with an ASAP2010 apparatus from Micromeritics. Before measurement, the samples were outgassed for at least 12 h under vacuum at 250 °C for silicas and at 120 °C for grafted silicas. The BET equation was applied to determine the surface area and C_{BET} parameter. Mercury porosimetry experiments were carried out with Micromeritics Autopore 9220 equipment. Samples were evacuated at room temperature. For all samples, at least two intrusion−extrusion cycles were carried out. A contact angle of 130° was used to calculate the pore size distribution using the Washburn equation. High resolution thermogravimetry under nitrogen gas flow was used to calculate the density of hydroxyl groups existing on the surface of the calcined samples (pure silica). Assuming that most OH groups are still on the surface after outgassing at 150 °C, it is possible to derive their amount from the mass loss between 150 and 850 °C. The TG analyses were achieved by a TGA Q500 apparatus from TA Instruments. The high-resolution program was followed from 30 to 850 °C. The epoxy density in the silicas was determined from thermogravimetric analysis (TGA) under an oxygen flow on a TG 209C analyzer (Netzsch Proteus). Adsorption isotherms of water vapor at 25 °C were determined with a homemade apparatus based on a symmetrical commercial vacuum microbalance from Setaram.[16] The samples were preliminarily evacuated under vacuum at 120 °C for 12 h.

Monte Carlo Simulations. *Models of Smooth and Rough Silica Surfaces.* To understand and characterize the depth of the surface rugosity obtained by pseudomorphic transformation, grand canonical Monte Carlo simulations of Ar adsorption on silica surfaces were performed. Different models of silica surfaces were considered. Model A is simply a regular silica surface without any surface rugosity (except the atomistic surface corrugation). Model B is a silica surface with a small cylindrical cavity in its center. Model C is a silica surface with a cylindrical cavity in its center having a constriction. The properties of the different pores considered in this work for models A, B, and C are reported in Table 2. We also report in Figure 1 transversal views of the silica surface without (model A) and with (models B and C) surface rugosity. For all surfaces, the square section of the silica surface is 10.695 × 10.695 nm^2. For model B, the cavity has a diameter D = 6.0 nm, and four cavity depths L = 1.0, 2.0, 3.0, and 6.0 nm have been considered. As a result, we considered for model B a total of four different silica surfaces B_L where L indicates the depth of the cylindrical cavity. For model C, we consider a cavity of a diameter

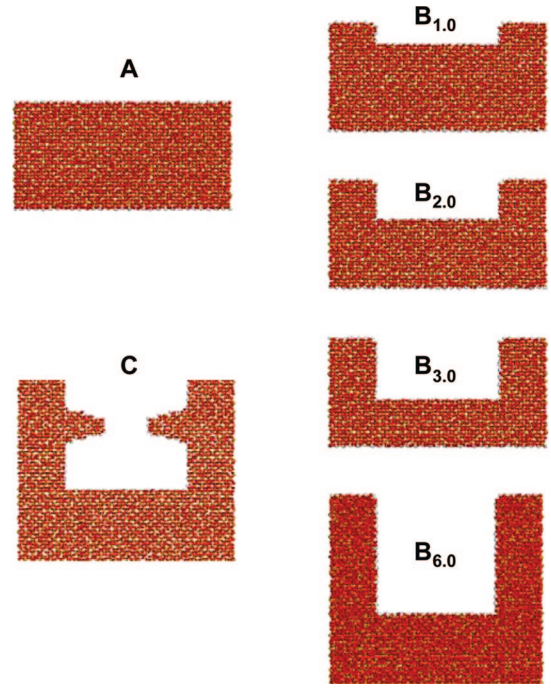

Figure 1. Transversal views of silica surfaces having a regular smooth (A) or a rough surface (B and C). For all surfaces, the square section of the silica surface is 10.695 × 10.695 nm^2. For model B, the cavity has a diameter D = 6.0 nm, and four cavity depths, L = 1.0, 2.0, 3.0, and 6.0 nm, have been considered. For model C, a cavity of a diameter D = 6.0 nm and a length L = 6.0 nm has a constriction along its axis of a diameter D_c = 2.0 nm and a length L_c = 2.0 nm.

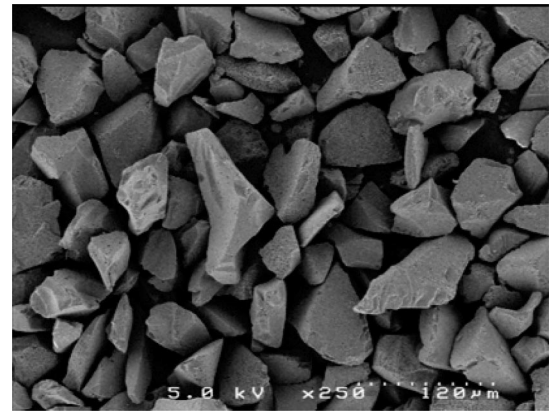

Figure 2. SEM picture of the parent FractoSil 800.

D = 6.0 nm and a length L = 6.0 nm. In addition, the cavity has a constriction along its pore axis of a diameter D_c = 2.0 nm and a length L_c = 2.0 nm.

The atomistic silica surfaces used in this work were generated according to the method proposed in ref 17. Coasne and Pellenq have shown that this technique can be used to prepare pores of various morphologies and/or topologies, such as cylindrical, hexagonal, ellipsoidal, and constricted pores.[18−20] To mimic the silica surface in a realistic way, we removed in a second step the Si atoms that are in an incomplete tetrahedral environment. We then removed all oxygen atoms that are nonbonded. This procedure

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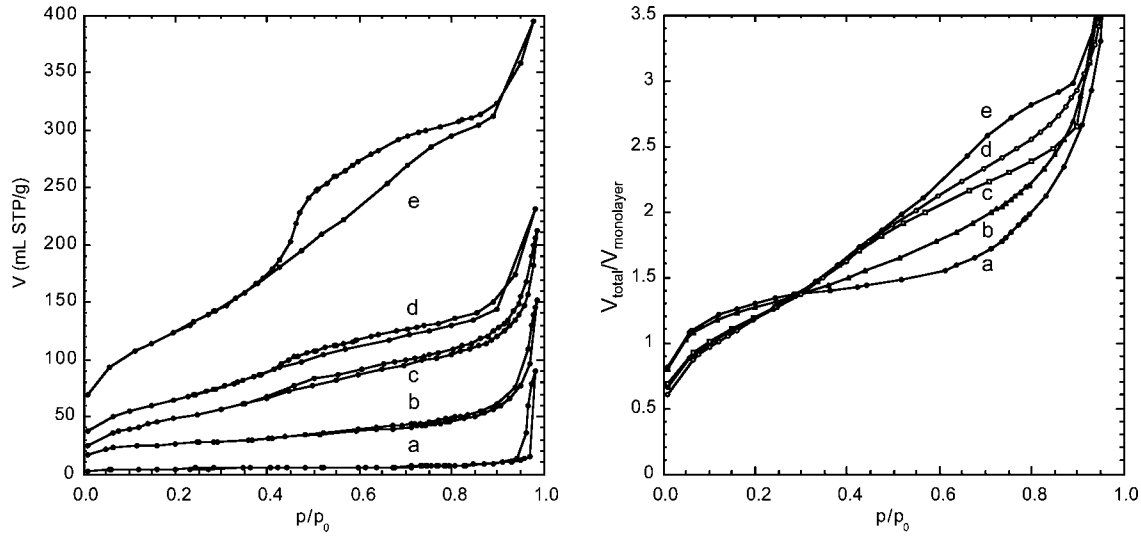

Figure 3. (left) Nitrogen sorption isotherms at 77 K of (a) the parent FractoSil 800 (square) and of the materials FractoSil PM obtained after 3 h (b), 4 h (c), 6 h (d), and 20 h (e) of reaction in alkaline medium in the presence of long-chain alkylammonium surfactants. (right) Same as shown in the left panel but total pore volume is divided by the monolayer volume to emphasize the step in adsorption.

ensures that the remaining silicon atoms have no dangling bonds and the remaining oxygen atoms have at least one saturated bond with a Si atom. Then, the electroneutrality of the simulation box was ensured by saturating all oxygen dangling bonds with hydrogen atoms. The latter are placed in the pore void, perpendicularly to the pore surface, at a distance of 1 Å from the closest unsaturated oxygen atom. Then, we displace slightly and randomly all the O, Si and H atoms to mimic an amorphous silica surface (the maximum displacement in each direction x, y, and z is 0.7 Å).

Grand Canonical Monte Carlo. We performed grand canonical Monte Carlo (GCMC) simulations of adsorption of argon at 77 K on the atomistic models of silica surfaces with and without surface rugosity. Comparison between simulation data for argon and experimental data for nitrogen adsorption at 77 K is made for all the surfaces considered in this work. Argon is easily used in simulation as it is a unique spherical atom, whereas nitrogen is an ovoid molecule composed of two atoms and can have different surface areas in function of its orientation. Nitrogen is nevertheless a standard in materials porosity characterization as it is a less expensive gas compared to argon. Qualitative comparison between argon and nitrogen adsorption is totally justified because these two adsorbates behave in a similar way when confined in porous materials. The GCMC technique is a stochastic method that simulates a system having a constant volume V (the pore with the adsorbed phase), in equilibrium with an infinite reservoir of particles imposing its chemical potential μ and temperature T.[21] The absolute adsorption/desorption isotherm is given by the ensemble average of the number of adsorbed atoms as a function of the pressure of the gas reservoir P (the latter is determined from the chemical potential μ according to the bulk equation of state for an ideal gas). The fluid/fluid interaction was calculated using a Lennard-Jones potential with $\varepsilon = 120$ K and $\sigma = 0.3405$ nm, which corresponds to the usual parameters for argon. The interactions between the fluid and substrate atoms were calculated using the PN-TraZ potential[22] developed for the adsorption of rare gases on siliceous materials.

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Results and Discussion

Silica Supports. The parent Fractosil 800 material appears as particles of 58 ± 25 μm in size (Figure 2) with a pore diameter of 80 nm and a low surface area of 16 m^2/g (Table 1). Direct application of an alkaline treatment to FractoSil 800 as described by Katoh et al.[6] led to the dissolution of 50% of the silica and a decrease of surface area down to 11 m^2/g. On the other hand, the full pseudomorphic transformation of FractoSil 800 using the procedure developed for silica beads and macroporous silica monoliths[7−12] led to a MCM-48 type of material with a surface area over 1018 m^2/g, a pore volume of 0.58 mL/g, and mesopores of 2.7 nm in diameter. The evidence for the pseudomorphic process was gained from SEM pictures and pore size distribution (PSD) determination (not shown). The latter shows that the transformed material retained the initial morphology and particle distribution of the parent silica. In spite of their unique textural properties, such materials may be however hardly applicable in the field of affinity ligand chromatography as a result of their small pore size.

To increase the surface area of FractoSil 800 while maintaining its original macroporosity and particle size, a treatment aiming at the generation of surface rugosity was attempted. This treatment takes advantage of the fact that the pseudomorphic transformation of a silica material under alkaline conditions in the presence of long-chain surfactants is kinetically controlled and is determined by the relative rates of the dissolution of the silica and of its reprecipitation and structuration around the surfactant micelles. Both processes can be controlled by adjusting the experimental parameters such as the composition of the reaction mixture.[23−26] In particular, we have shown

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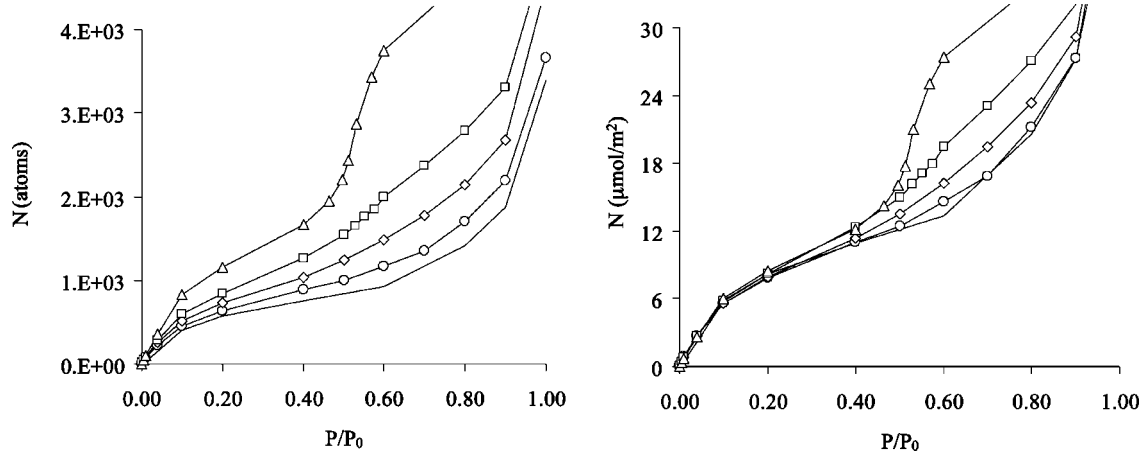

Figure 4. (left) Simulated adsorption isotherm at 77 K for argon on smooth and rough silica surfaces. The solid line is for the smooth silica surface (model A). The circles, diamonds, squares, and triangles are for the silica surfaces $B_{1.0}$, $B_{2.0}$, $B_{3.0}$, and $B_{6.0}$, respectively. P_0 is the bulk saturating vapor pressure. (right) Same as shown in the left panel but the adsorbed amounts are in μmol per unit of surface area.

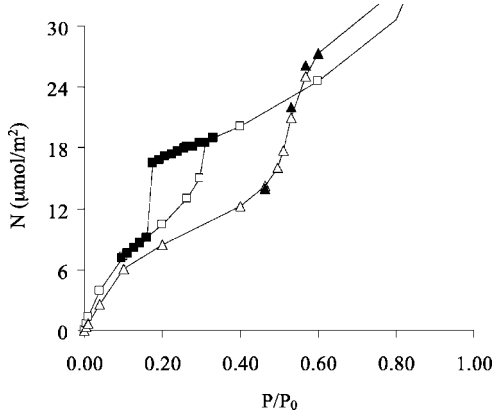

Figure 5. Simulated adsorption isotherm at 77 K for argon on rough silica surfaces. The triangles are for the rough silica surface $B_{6.0}$ while the diamonds are for the rough silica surface with a constriction C. The open and closed symbols are for the adsorption and desorption data, respectively. The adsorbed amounts are in μmol per unit of surface area. P_0 is the bulk saturating vapor pressure.

previously that the addition of TMB to the CTAB templating mixture leads to a significant increase in the rate of precipitation of a hybrid silica−organic mesophase at the surface of the particles.[23] The formation of such a mesophase prevents the silica particles from further dissolution by limiting the diffusion of the alkali inside the bulk of the grains.

Nitrogen Adsorption Measurements. Figure 3 and Table 1 report the nitrogen isotherms and textural characteristics of materials obtained after pseudomorphic transformation of parent FractoSil 800 particles at 90 °C in a mixture of composition 1 SiO_2/0.075 C_{16}TAB/0.3 NaOH/0.02 NaI/0.11 TMB/0.15 decane/40 H_2O, for 3, 4, 6, and 20 h. After calcination, the nitrogen adsorption/desorption isotherms of the amorphous FractoSil PMs material (Figure 3) reveal a continuous increase in adsorption with the pseudomorphic treatment duration, which corresponds to an increase of surface area from 16 m^2/g for FractoSil 800 to 94, 178, 235, and 456 m^2/g for FractoSil PM after pseudomorphic treatment of 3, 4, 6, and 20 h, respectively. FractoSil PM obtained after 3 h of reaction shows an adsorption isotherm very close in terms of surface polarity (similar C_{BET}) to the parent FractoSil. These high values of the C_{BET} parameter (ca. 300), too high for a nonporous silica surface which stands classically around $C_{BET} \sim 100$, suggest either a small contribution of microporosity or the presence of defects generating a high concentration of silanols. For materials obtained after 4 or 6 h of reaction, the surface increases to 178 and 235 m^2/g, respectively, and a very slight hysteresis appears. However, the nitrogen adsorption/desorption isotherms are mostly reversible. By comparing the slope of the isotherms in the representation of pore volume/monolayer volume, meaning the amount of molecules adsorbed per surface unit (Figure 3B), to emphasize the adsorption step, no real step due to pore filling is observed, but a large increase of pore volume on a large range of pressure (equivalent to pore domains from 3 to 10 nm) is observed. For the material obtained after 20 h of reaction, a real step in adsorption is observed as well as the presence of a hysteresis loop characteristic to the presence of real pores with a broad distribution of pores in the range 3−10 nm, and this material presents a hysteresis. FractoSil PMs obtained after 4, 6, and 20 h of reaction exhibit C_{BET} parameters in accordance with a surface polarity expected for MCM-41 type materials (typically $C_{BET} = 60-90$) suitable for high protein coupling.[15] To understand the origin of the increase of surface area in FractoSil PM, with or without the apparition of step in the isotherm and with or without the presence of an hysteresis loop, simple geometrical calculations of surface roughness as well as adsorption simulations have been performed.

Rugosity and Surface Area. Starting from a nonporous or macroporous surface having a low surface area, the creation of rugosity will increase the surface area of the parent material. Starting with an initial surface area $S_0 = \pi r^2$ corresponding to a disk of radius r, the increased surface area S_1 obtained after replacing the disk by a single cylindrical pore of radius r and depth h is

$$S_1 = \pi r^2 + 2\pi hr$$

From this equation, one obtains that the increase factor $n = S_1/S_0$ in the surface area is

$$n = 1 + 2h/r$$

Therefore, if the external surface is neglected, the surface of the material will be 5 times that of the nonporous material for

Table 3. Textural and surface characteristics of the parent FractoSil 800 and of the modified FractoSil PM materials

material	density (g/cm^3)	surface area (m^2/g)	particle size distribution (μm)	3-glycidoxy propylsilane ($\mu mol/g$)	(molecule/nm^2)
FractoSil 800	0.75	16	58 ± 25	98	2.57
			Pseudo 90 °C, 4 h		
PM-130	0.52	130	46 ± 25	188	0.85
PM-96	0.58	96	53 ± 25	185	1.14
PM-160	0.49	160	47 ± 25	209	0.77
PM-132	0.46	132	46 ± 25	173	0.78
PM-117	0.43	117	43 ± 25	219	1.11
			Pseudo 90 °C, 6 h		
PM-298	0.41	298	55 ± 25	483	0.98
PM-236	0.41	236	44 ± 25	391	1.00
PM-218	0.43	218	47 ± 25	325	0.90
PM-235	0.41	235		290	0.75

$h = 2r$ and 9 times for $h = 4r$. In general, the surface area of a material can be significantly increased if the depth of the rugosity is at least of the same range of its diameter. From the models of silica surfaces used in our Monte Carlo simulations (Figure 1), we found that a cylindrical rugosity of a diameter of 6 nm on a 114 nm^2 planar substrate increases the surface area from 133 up to 227 nm^2 for a depth of 1−6 nm. After subtraction of the nonporous surface (86 nm^2), the surface of the pore increases from 28 up to 168 nm^2. Figure 4 shows the adsorption isotherm at 77 K on smooth (model A) and rough silica surfaces (model B). An increase in the slope of the adsorption isotherm is observed in the adsorption isotherm between $P = 0.4P_0$ and $0.6P_0$. The latter corresponds to the continuous filling of the 6.0 nm cylindrical cavity in the planar substrate. The increase in the slope becomes more marked as the depth of the cylindrical cavity increases. In particular, a step relative to pore filling by capillary condensation is observed for the pore with a depth equivalent to the pore diameter ($h = D = 6.0$ nm). The increase in the slope of the adsorption isotherm is even more pronounced as the adsorbed amounts are reported per unit of surface area (Figure 4). These results show that surface rugosity on silica substrates can be assessed using gas adsorption, even for low rugosity depths. Adsorption/desorption isotherms on silica substrates without surface rugosity (model A) or with a regular cylindrical rugosity (model B) were found to be reversible. This is consistent with the fact that condensation and evaporation on pores closed at one end is expected to be reversible[27] (both the pore filling and emptying proceed through the displacement at equilibrium of a hemispherical meniscus along the pore axis). In contrast, in the case of model C (cylindrical surface rugosity with a constriction), the adsorption/desorption isotherm exhibits a large hysteresis loop (Figure 5). The latter is due to the entrapment of the confined fluid upon desorption which empties through cavitation or pore blocking at a pressure much lower than that of capillary condensation (see ref 20 for a detailed discussion on adsorption and desorption of simple fluids in pores with constrictions).

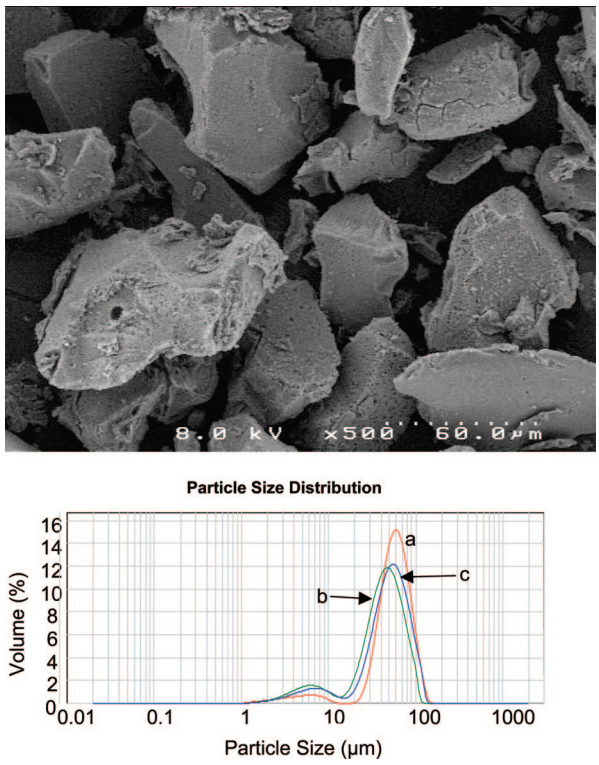

Figure 6. SEM picture of FractoSil PM obtained after 6 h of reaction in alkaline medium in the presence of long-chain alkylammonium surfactants and particle size distribution of the parent FractoSil 800 (a) and of FractoSil PM obtained after 4 h (b) and 6 h (c).

By comparing the experimental results with the simulations, one can conclude that FractoSil PMs (obtained after 4 and 6 h of reaction time) exhibiting a continuous increase of pore volume in the adsorption branch have a surface characterized by a rugosity with a diameter comprised between 3 and 10 nm and a depth lower than 10 nm as no real step is observed. The small hysteresis observed upon desorption is due to the presence of few pores with constriction. Their volume is, however, very small, which supports the hypothesis that the textural changes result essentially from a surface alteration of the FractoSil 800 particles. The hypothesis of a process limited to the surface by the diffusion of the alkali is confirmed by the experiment run at longer reaction time (FractoSil PM after 20 h reaction), for which the shape of the adsorption isotherm is significantly modified and shows the apparition of a step and hysteresis loop characteristic of mesopore formation in the same size range (3−10 nm) with length higher than 10 nm and the presence of constrictions. FractoSil PM obtained after 4 and 6 h of reaction appears as the most suitable support, with no mesopores and a surface roughness of 10 nm wide maximum and less than 10 nm depth.

Preparation Reproducibility. The reproducibility of the preparation of the FractoSil PM materials was also tested.

(27) Gelb, L. D. *Mol. Phys.* **2002**, *100*, 2049.

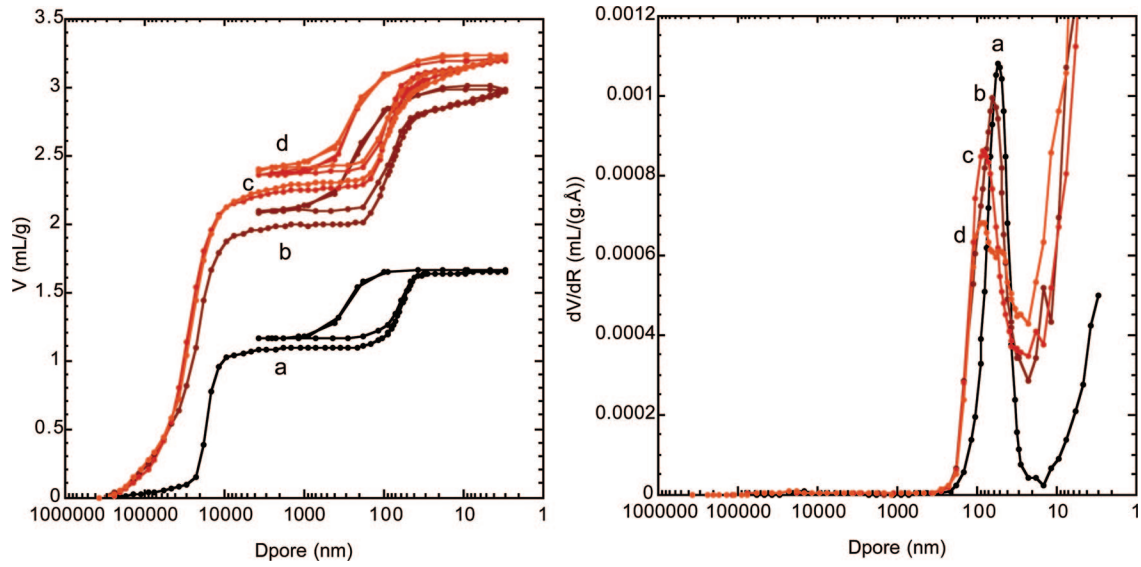

Figure 7. (left) Mercury porosimetry isotherms for FractoSil 800 (a) and of FractoSil PM obtained after 3 h (b), 4 h (c), and 6 h (d) of reaction in alkaline medium in the presence of long-chain alkylammonium surfactants. (right) Pore size distribution and in inset mercury porosimetry isotherms corresponding to the intrinsic macropore corrected for interparticular pore volume.

Table 4. Characterization of the Parent FractoSil and of the Modified FractoSil PM Materials: Silanol Density and Water Adsorption Capacity

material	surface area (m^2/g)	water weight loss 150−850 °C (mg/g silica)	SiOH (mmol/g)	SiOH ($/nm^2$)	water ads at monolayer coverage (H_2O/nm^2)
FractoSil 800	16	3.6	0.40	15.1	4.0
PM-82	82	6	0.67	4.89	3.3
PM-133	133	9	1.00	4.52	2.4
PM-178	178	11.2	1.24	4.21	1.9
PM-236	236	12	1.33	3.40	2.4

Table 3 reports the surface area of two series of materials produced by reacting the parent FractoSil 800 particles at 90 °C for 4 h and 6 h. Each data point corresponds to an experiment performed starting from 15 g of FractoSil 800. In spite of some dispersion of the data it appears that the surface area of the parent silica is increased to 130 ± 50 m^2/g and to 250 ± 50 m^2/g after 4 and 6 h of reaction, respectively. The particle size and particle size distribution (Table 3, Figure 6) of the original silica particles have not been modified by the treatment, indicating that the reaction actually consisted of a pseudomorphic transformation. After calcination 10 ± 2 g of material are obtained, corresponding to a silica yield of 70 ± 10%. Accordingly, all the modified solids exhibited a lower density that the parent FractoSil.

Mercury Porosimetry Measurements. The preservation of the size of the macropores of the parent FractoSil has been ascertained by mercury porosimetry measurements performed over FractoSil PMs obtained after 3, 4, and 6 h of reaction (Table 1, Figure 7). The isotherms of FractoSil PMs are similar to those of the parent FractoSil with a first step in intrusion corresponding to interparticular porosity at around 20 μm, in accordance with the preservation of the particle size and shape, and a second step at around 70−100 nm indicating the preservation of the initial macroporosity. In the low pressure range (large pore size), the intrusion curves show a different behavior of the fluid to fit the pore shape between the parent material and the other samples (this effect may be accentuated by the fact that a between 2 and 5 times less amount of powder has been used for FractoSil PM analysis in comparison with the amount for parent FractoSil). This may be related to the apparent increase of roughness with chemical treatment that can be observed by comparing SEM images of Figures 2 and 6. This surface roughness is confirmed by the mercury intrusion curves below 10 000 nm, which show an increase of pore volume more pronounced for FractoSil PM samples. By comparing the intraparticle macroporosity (pore volume) of the materials (Figure 7, Table 1), one can see that a higher pore volume has been found for FractoSil PM compared to parent FractoSil. This is related to the fact that the macropore size distribution given in Figure 7 shows a small increase in mean pore size when increasing the pseudomorphic treatment time: from 79 nm for the parent FractoSil up to 100 nm for FractoSil PM obtained after 4 and 6 h (Table 1). The mercury extrusion branches for the modified samples are very similar, but the hysteresis widths are narrower than that for Fractosil 800, revealing a smaller cavity size.[28] This suggests that the pseudomorphic transformation has made the pores more cylindrical, although rugosity has been created (as shown by the increased surface area that is 10−15 times higher than that of the parent silica).

The apparent increase of pore volume for pores in the range around 100 nm does not mean that the pseudomorphic transition created many new pores in that range. Indeed, if this treatment had led to the creation of new pores by the dissolution of silica in another pore size range (here in the mesopore range 3−10 nm) accessible (or not) to mercury at another pressure, the apparent volume of intruded mercury per mass of adsorbent would increase. It is possible to quantify this effect by the following reasoning. Let us call $V_{p(macro)}^{i}$ the initial pore volume of 1 g of the parent material in the macropore range (here around 100 nm). If the

(28) Coasne, B.; Galarneau, A.; Di Renzo, F.; Pellenq, R. J. M. *J. Phys. Chem. C* **2009**, *113*, 1953.

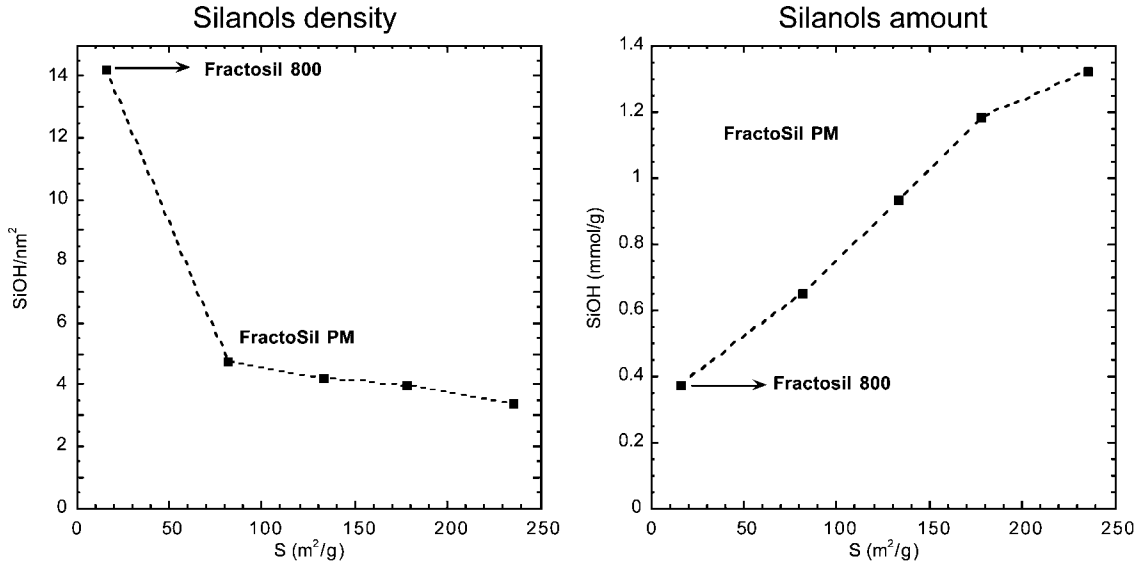

Figure 8. Density of silanol groups in (left) SiOH/nm^2 and (right) mmol SiOH/g determined by TGA as a function of the surface area of FractoSil PM.

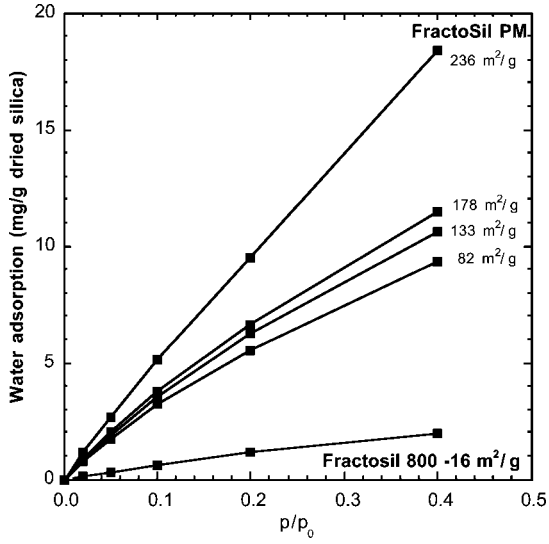

Figure 9. Water sorption isotherms at 25 °C on FractoSil 800 and FractoSil PM of different surface area.

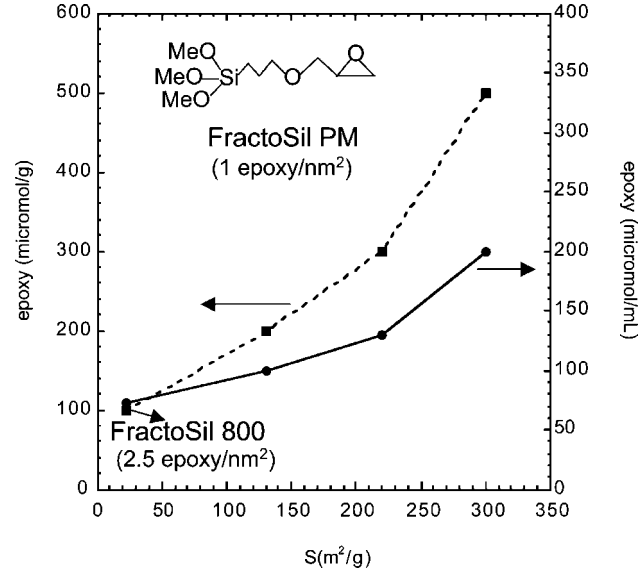

Figure 10. Amounts per unit of weight and of volume of grafted glycidoxy propylsilane groups on FractoSil 800 and FractoSil PMs as a function of their surface area.

pseudomorphic modification created a mesoporous volume by dissolution of x g per g of silica, then the specific volume of the produced sample in the macropore range would be

$$V_{p(macro)}{}^{f} = V_{p(macro)}{}^{i}/(1-x)$$

From a zoom in the macropore size range in Figure 7A, one can observe that $V_{p(macro)}{}^{i}$ is about 0.55 mL/g whereas for the Fractosil PM samples the intrusion step around 100 nm leads to a $V_{p(macro)}{}^{f}$ of about 0.8 mL/g, which leads to a dissolution value $x \sim 0.3$ g/g, in agreement with the 70% silica yield quoted above.

By mercury intrusion, a small proportion of smaller pores is also identified as evidenced by a continuous increase in pore volume corresponding to pore sizes from 10 down to 3 nm and accounting for ~0.2 mL/g of the total pore volume (Figure 7). This shows that the surface rugosity can also be evidenced by mercury intrusion. Note that the pore volume found by nitrogen adsorption for FractoSil PM is also about 0.2 mL/g (Figure 3A).

Surface Properties. The nature of the surface of the parent FractoSil 800 and of the modified materials was investigated by TGA measurements. The amount of silanols was calculated from the weight loss recorded between 150 and 850 °C on samples previously calcined at 550 °C to remove the surfactant and then allowed to rehydrate under ambient conditions. Results are reported in Table 4 and in Figure 8. Upon increasing the surface area, the density of silanol groups decreases from 15 SiOH/nm^2 for the parent silica down to 4−5 SiOH/nm^2 for the treated materials. We have previously shown that pseudomorphic transformation of high surface area porous silica gels in the presence of long-chain alkylammonium cations reduces the hydrophilic character of the surface through a decrease of the density of silanol groups from approximately 5 SiOH/nm^2 for amorphous silica down to approximately 2 SiOH/nm^2 for micelle templated silicas.[11] Although the transformation is not complete here, the high density of silanol found on the parent FractoSil 800

suggests the presence of internal silanols groups. It is clear from our data that the surface of the modified materials differs significantly from that of the parent FractoSil. The former is less hydrophilic than the latter. Such a conclusion is supported by the water adsorption data reported in Figure 9: the water adsorption isotherms reveal that larger amounts of water are adsorbed per weight bases upon transformation. However, because of the increase in the surface area, it turns out that the water coverage per unit of surface decreases on the FractoSil PM materials. Indeed, by applying the BET theory to the water adsorption isotherms one may calculate the monolayer water coverage. The density of water molecules at monolayer decreases from 4 to 2 H_2O/nm^2 (Table 4) from the initial FractoSil to PM-236, respectively. Such a value of 2 H_2O/nm^2 is equivalent to that reported for MCM-41 materials,[11] suggesting that the surfaces of the materials are very similar. It probably means that part of the silanols determined by TGA on Fractosil PM are internal like in the parent material.

Such a finding is particularly relevant as it has been shown that the combination of a large surface area and of a low silanol density in large-pore MTS materials allowed for a more effective protein coupling compared to traditional silicas by minimizing the competing interactions with silanols.[15]

Surface Functionalization. Calcined FractoSil 800 and modified FractoSil PM have been grafted with trimethoxyglycidoxysilane for future immobilization of proteins or biomimetic ligands. The density of the epoxy group for all modified FractoSil PMs was 0.90 ± 0.15 epoxy/nm^2, whereas 2.5 epoxy/nm^2 has been grafted over the initial FractoSil 800 (Table 3). However, once again, the positive compensation resulting from the surface area increase leads to a 5-fold (on weight bases) from 0.10 to 0.50 mmol/g or 2-fold (on volume bases) from 0.10 to 0.20 mmol/mL column increase of the amount of anchored epoxy groups (Figure 10). For comparison purposes, a polymeric phase proposed as affinity chromatography stationary phase by Merck (Fractogel EMD epoxy (M)) features a ligand density of 0.5 to 1 mmol/g or 0.14 to 0.28 mmol/mL in volume. This indicates that FractoSil PM could constitute an alternative to polymeric phases in affinity chromatography. Furthermore, FractoSil PM type supports are mechanically more stable than polymeric phases (as witnessed by the reversibility of the mercury intrusion experiments performed by applying pressures up to 4000 bar (Figure 7) whereas polymeric are stable only until 20 bar) and have no disturbing swelling properties. As preliminary results, 17 mg of protein A/mL (protein A is used to selectively bind human antibody IgG) has been coupled on FractoSil PM-100 and results in a dynamic IgG affinity of 22 mg IgG/mL. These values compete favorably with those achieved with most polymeric phases and those reported by by Katoh et al. for transformed silicas, which stand at only 8 mg of protein A/mL.[6]

Conclusion

The surface area of a macroporous silica support suitable for affinity chromatography appearing in the form of 50 μm particles has been increased by applying a dissolution/reprecipitation process in the presence of long-chain alkylammonium surfactants previously developed for the pseudomorphic transformation of amorphous silica gels into ordered MCM-41 and MCM-48-type materials. By limiting the transformation process to the surface of the particles, through an adjustment of the experimental conditions, we have been able to generate a surface roughness consisting in cavities of diameter 3−10 nm with a maximum depth of 10 nm, without modification of the macropore size (80 nm), pore volume, particle size, and morphology of the parent support. Surface areas in the range 100−300 m^2/g have been thus achieved.

In addition, the pseudomorphic treatment leads to a transformation of the nature of the silica surface allowing a large amount of epoxy groups to be grafted. The procedure introduced should offer the possibility to prepare materials with a high protein coupling capacity. Such silica supports could therefore compete with traditional polymeric phases used in affinity chromatography and for the separation of large molecules.

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