Hyperbranching Surface Polymerization as a Tool for Preferential Functionalization of the Outer Surface of Mesoporous Silica[†]

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A highly flexible method for selective functionalization of mesoporous silica is presented, where the key step is the preferential growth of hyperbranched poly(ethylene imine), PEI, on the outer surface of the material. Here, the monomer aziridine is polymerized in the presence of silica particles in which the surfactant used as structure-directing agent has not yet been extracted from the mesopores. Furthermore, carboxylic acid functionalities can be introduced preferentially to the outer part of the PEI layer by succinylation, and the relative concentration of carboxylic acid to amino groups in the PEI layer can be rationally controlled. This makes it possible to fine-tune the surface charge of the particles for a given application independently from the thickness of the surface layer, which is expected to offer advantages in applications where further surface functionalization is needed, in combination with molecular gate properties and/or a high dispersion stability. The introduced method also allows the synthesis of materials with antagonist (Brønsted) acidic−basic and hydrophobic−hydrophilic properties. The surface functions are accessible and covalently linked to the particle, which is important for example in order to ensure that targeting functions do not detach from the particle during targeted drug release applications, which is not necessarily the case when the surface layer is only adsorbed to the particle surface.

Introduction

Mesoporous silicas synthesized using supramolecular surfactant aggregates as structure-directing agents[1] have attracted lots of recent interest in applications related to immobilization and release of active substances, especially drugs.[2,3] In contrast to most polymer-derived matrices, these highly porous materials exhibit a narrow pore size distribution in combination with a high specific pore volume. This feature could ease an even incorporation of organics into the structures and, further on, provide a controlled release. Furthermore, the high adsorption capacity of this class of materials facilitates a high loading of drug. A much desired property of such systems is the possibility to open and close the pores upon external stimuli in order to achieve "release on demand" behavior. Several different approaches have recently been proposed where the outer particle surface or the pore mouths have been functionalized, with the aim of achieving an externally triggerable release,[4–10] as recently reviewed.[3] The outer surface can be functionalized by temperature-responsive, photocontrolled, or pH-responsive polymers or by chromophores that undergo cis–trans transitions upon irradiation with light.[7–9] Furthermore, erosion of polymer layers or corrosion of inorganic particles present in the pores or close to the pore mouths can also be used for this purpose.[2a]

However, selective functionalization of the particle surface is not only important for introducing responsive molecular gate functionality into porous particulate systems. Often the particle surface needs to be modified in order to prevent particle aggregation in solution under conditions relevant for the target application. Furthermore, targeting of specific cells in drug delivery applications requires that additional functions, such as specific antibodies, are attached to the particle surface. If the surface groups that are present on the outer surface are different from those present on the pore walls, a

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selective attachment of the targeting functions on the outer particle surface is easier to achieve. The linking chemistry is often relying on amine−carboxylic acid interactions, and thus the presence of amino or carboxylic acid functions on the outer particle surface is of special interest. Bifunctional mesoporous materials with coexisting antagonist acidic and basic groups have been reported before, but here the groups are typically distributed evenly throughout the materials.[11] The highest level of flexibility would be achieved if the outer surface of the particles and the pore surface could be functionalized independently from each other, as then the adsorbent–adsorbate interactions and the outer surface of the particles could be optimized separately for a given application. In this respect, the recent study by Casasús et al.[5] is especially interesting. They reported the synthesis of a selectively functionalized mesoporous silica with pH-responsive amino groups preferentially located at the pore outlets, based on a two-step procedure. In the first step, thiol groups were introduced by co-condensation, using $(EtO)_3Si-CH_2CH_2CH_2SH$ as the organosilane. However, before template removal, the material was treated with $(EtO)_3Si-(CH_2)_3(NHCH_2CH_2)_2NH_2$ in an attempt to selectively functionalize the outer surface of the particles. After surfactant removal, the material exhibited both pH- and anion-controlled molecular gate properties, where the pores were open under pH conditions where the amino groups were not protonated. Coulombic repulsion between charged amino groups rendered the polyamine to adopt a more open structure, which served to keep the pores closed to the outside, as demonstrated by pH-dependent adsorption of a blue squaraine dye from solution, which selectively bleach upon contact with the mercapto groups on the mesopore walls. Inspired by these results, we have extended this approach to a range of mesoporous silicas, where the outer particle surface is preferentially functionalized by surface-grown poly(ethylene imine) (PEI), which was obtained by acid-catalyzed hyperbranching surface polymerization of aziridine. Hyperbranching surface polymerization is a promising method for amino functionalization of mesoporous silica,[12–16] as the surface concentration of amino groups on the mesopore walls can be much higher than what is normally achieved by silanization.[15] The quantity, and thus also the thickness, of the PEI layer can be controlled simply by changing the aziridine/silica ratio in the surface functionalization step. Moreover, we demonstrate that a hydrophobized mesoporous silica material can be fully redispersed in aqueous media by preferential functionalization of the outer surface of the silica particles by PEI. We also demonstrate that carboxylic acid functions can be introduced to the outer part of the PEI layer, thus allowing full flexibility for further functionalization of the particles by standard linking procedures especially important for bioapplications. The introduced methodology also offers a means for fine-tuning of the effective surface charge of the particles at a given pH, which is one of the key parameters for dispersion stability. Finally, this could open up new possibilities for release on demand in biological systems, as the local pH in different tissues may vary by several units.

Experimental Section

Synthesis of SBA-3 Substrate Material. Mesoporous SBA-3 silica was synthesized as proposed by Huo et al.[17] Cetyltrimethylammonium bromide, $C_{16}TAB$ (Aldrich), was used as structure-directing agent and tetraethoxysilane (TEOS, 98%, Aldrich) as silica precursor, and the synthesis of the following molar ratio was employed: 0.12/9.2/130/1 for $C_{16}TAB/HCl/H_2O/TEOS$. The synthesis mixture was stirred for 3 h at room temperature (RT), filtered, rinsed with distilled water, and dried at 363 K for 24 h.

Synthesis of SBA-15 Substrate Materials. Mesoporous silica SBA-15 parent materials were synthesized according to Choi et al.[18] using a triblock copolymer, Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, Aldrich), as structure-directing agent. TEOS was used as silica precursor with molar ratios of $n_{TEOS}/n_{P123} = 60$, and the synthesis mixture was aged for 24 h at 368 K, filtered, and dried at 323 K for 24 h.

Co-condensed amino-SBA-15 silica (CC-SBA-15) was synthesized in a similar manner according to the procedure described by Wang et al.[19] using triblock copolymer P123 as structure-directing agent and 3-aminopropyltriethoxysilane (APTES, 98%, ABCR) as organosilane. The synthesis mixture had a composition of TEOS/APTES/HCl/P123/H_2O: 0.9/0.1/6.1/0.017/165 and was stirred for 20 h at 35 °C and subsequently aged at 368 K for 24 h, filtered, and dried at 323 K for 24 h.

Functionalization. Aziridine was synthesized from aminoethylsulfuric acid (Aldrich) according to the procedure described by Allen et al.[20] Surface polymerization of aziridine to yield surface-grown poly(ethylene imine) (PEI) was performed with toluene as solvent, in which the substrates were suspended after being carefully vacuum-dried and sealed under vacuum and subsequently subjected to argon atmosphere. Catalytic amounts of acetic acid were added under stirring, after which aziridine was added in amounts ranging from 0.2 to 0.55 mL per gram of SiO_2. The suspension was refluxed under argon atmosphere overnight at 348 K, filtered, washed with toluene, and dried in vacuo at 323 K.

Succinylation was performed according to a procedure described by Schiestel et al.[21] PEI-functionalized materials were suspended in tetrahydrofuran (THF, 99.9+%, Aldrich), and succinic anhydride was added in excess and sonicated. The suspension was stirred overnight, filtered, washed with THF, and dried in vacuo at 323 K. A schematic representation of the different functionalization steps

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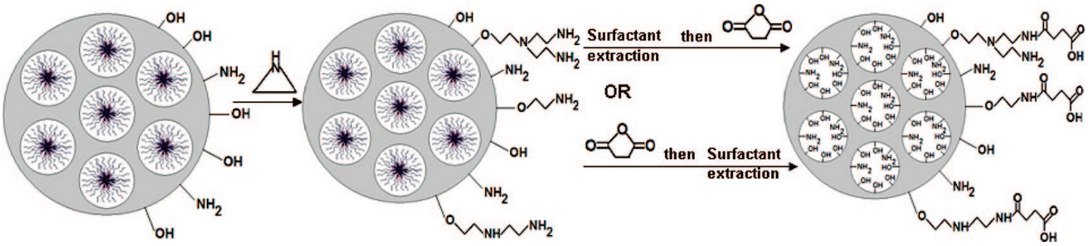
Scheme 1. Schematic Representation of the Steps Involved in the Selective Functionalization of SBA-15 with Amino Groups on the Pore Walls and Carboxylic Acid and Amino Groups Preferentially Located on the Outer Surface of the Particles



used for the selective functionalization of SBA-15 by amino groups on the internal pore walls and amino and carboxylic groups on the outer particle surface is shown in Scheme 1.

Hexamethyldisilazane (HMDS) treatment[22] of the silanols of postextracted PEI-SBA-15 and reference material standard SBA-15 was performed in vapor phase at 323 K overnight. The resulting material was dried in vacuo at 323 K.

Extraction. Extraction was performed in ethanol under reflux at 340 K overnight as a suspension of 0.3 g of material per 100 mL of ethanol with or without added (0.2 g) HCl. The materials were filtered, washed with ethanol, and dried in vacuo at 323 K. When HCl was used in the surfactant extraction step, TMAOH treatment was subsequently performed in order to avoid the presence of $R-NH_3Cl$ groups in the amino-functionalized materials.

Characterization Methods. *X-ray Diffraction.* X-ray diffraction (XRD) measurements were performed using a Kratky compact small-angle system (Hecus Braun, Austria). The system is equipped with a position-sensitive detector consisting of 1024 channels of 55.5 μm width each. A Seifert ID-300 X-ray generator, operating at a maximum intensity of 50 kV and 40 mA provided the Cu Kα radiation at $\lambda = 1.542$ Å. A Ni filter was used to remove Kβ radiation, and a W filter protected the detector from the primary beam. The sample-to-detector distance was 267 mm. The sample holder was kept under vacuum during the measurements in order to minimize the background scattering from air.

Nitrogen Sorption at 77 K. Nitrogen physisorption measurements were performed at 77 K using an ASAP 2010 sorptometer (Micromeritics). The functionalized materials were outgassed at 323 K prior to the measurements in order not to degrade the polymer layer.[23] The NLDFT kernel (Autosorb 1 software, Quantachrome Instruments) developed for silica exhibiting a cylindrical pore geometry[24] was used for pores size and pore volume determination.

Thermogravimetric Analysis. Thermogravimetic analyses were performed using a Netzsch TGA 209. Each sample was compared to a reference sample measured with the same background run and temperature program, heating up to 1173 K at a heating rate of 10 K/min in air. Nitrogen was used as protective gas. The TGA runs were analyzed by the Netzsch Proteus Thermal Analysis Software, v. 4.3.1. The sample and the reference were first normalized against the dry weight, and then the reference was subtracted from the sample and the mass loss normalized to the mass of the substrate to yield the mass increase (in wt %) for the functionalization step in question.

Electrokinetic Titrations. The IEP titrations were performed using a Malvern ZetaSizer Nano-ZS coupled to a MPT-2 Titrator unit. The zeta-potential was measured as a function of pH by titrating with 0.1 M HCl and NaOH at 298 K. The samples were suspended in deionized water yielding a suspension of about 0.1% m/V and sonicated for 1 min in a FinnSonic m08 ultrasound bath. The data were monitored and analyzed using Malvern Dispersion Technology software v. 4.20.

Salicylic Acid Adsorption. pH-dependent salicylic acid (SA) adsorption was performed from aqueous solutions with an initial concentration of 15 mM. The pH was adjusted prior to addition of the functionalized silicas in order to avoid volume changes leading to dilution of SA. Suspensions of 5 mg/mL of solvent was prepared, stirred overnight, and centrifuged at 3000 rpm for 15 min. The SA content in the supernatant was measured using a Shimadzu UV–vis spectrophotometer reading at wavelength $\lambda = 303$ nm.

Results and Discussion

All materials discussed below were characterized by X-ray diffraction, N_2 sorption at 77 K, thermogravimetric analysis, and electrokinetic titration, and the main relevant parameters are summarized in Table 1.

PEI Functionalization of Mesoporous SBA-3 Material. Selective functionalization of the outer surface of mesoporous silica with PEI is first demonstrated for SBA-3, which is a mesoporous silica synthesized under acidic conditions using cetyltrimethylammonium bromide as the structure-directing agent. SBA-3 was chosen as the surfactant can easily be removed by postextraction. The XRD pattern measured for the SBA-3 material after extraction of the surfactant is shown in Figure 1, and it can be indexed to *p*6*m* symmetry, in agreement with its 2D hexagonal arrangement of cylindrical pores. The N_2 sorption isotherm of the same material is shown in Figure 2. The mesopore diameter calculated on the basis of the NLDFT kernel developed for cylindrical pores was 3.8 nm. The aziridine polymerization was carried out on dried as-synthesized SBA-3 before the surfactant extraction step. Thus, the growth of PEI should preferentially occur on the outer surface of the SBA-3 particles. The nitrogen sorption isotherm of the PEI-functionalized SBA-3 material is also shown in Figure 2. The PEI content of this material was 8 wt %. The mesopore diameter remains virtually the same after PEI functionalization, although the BET specific surface area and the total pore volume naturally decrease upon PEI functionalization as a consequence of increasing mass of the material (see Supporting Information). However, the decrease in specific surface area and pore volume upon functionalization is higher than that which would be expected for functionalization of only the outer surface of the particles, which seems to suggest that some functionalization of the mesopore surface has also occurred. However, additional effects that could contribute to this observation are the possibility of some remaining surfactant

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Table 1. Characteristics of the Materials under Study Obtained by Nitrogen Sorption at 77 K, Thermogravimetric Analysis, and Electrokinetic Measurements

sample	A_s (BET) [m^2/g]	d_p [nm]	V_p [cm^3/g]	C value	new funct[a] [wt %]	total org[b] [wt %]	IEP
SBA-3 extracted	1431	3.8	1.08	44			4.2
PE-PEI-SBA-3	1165	3.8	0.80	41	8.0	8.0	9.7
SBA-15 extracted	1109	7.9	1.16	107			2–4
PE-PEI-SBA-15	428	7.9	0.71	42	28.2	28.2	9.6
HMDS-PEI-SBA-15	330	7.6	0.51	24	2.8	30.2	9.5
HDMS-SBA-15[c]	696	6.8	0.89	28	1.5	1.5	N/A
CC-SBA-15	753	7.0	0.90	67		7.5	7.9
PE-PEI-CC-SBA-15	345	7.0	0.57	30	21.5	27.4	10.4
PE-succ-PEI-CC-SBA-15	220	7.0	0.36	60	15.5	38.7	6.5
PEI-CC-SBA-15[d]	176	6.1	0.29	35	20.1	26.1	10.6

[a] The mass increase recorded for the functionalization step in question from TGA. [b] Total organic content of the material as estimated from TGA. [c] Standard SBA-15 was used as substrate, with critical characteristics A_s(BET) = 797 m^2/g, d_p = 7.3 nm, V_p = 0.93 cm^3/g, and C = 129. [d] From ref 15. Code for material names: PE = postextracted; succ = succinylated; PEI = PEI-functionalized; CC = co-condensed amino-SBA-15. The materials are indexed so that the last performed functionalization step appears first, etc.

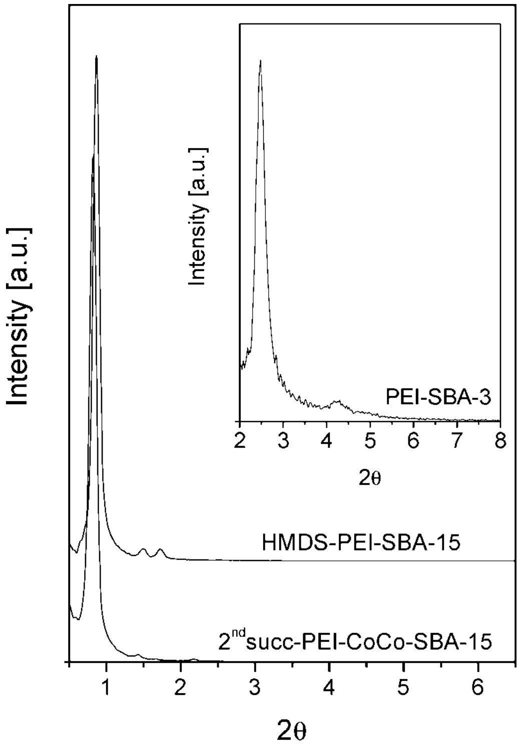

Figure 1. Powder XRD patterns of selected functionalized materials. The *d*-spacing of the PEI-SBA-3 material was 3.6 nm, 10.4 nm for the HMDS-PEI-SBA-15 material, and 10.6 nm for the twice succinylated 2nd succ-PEI-CC-SBA-15 material.

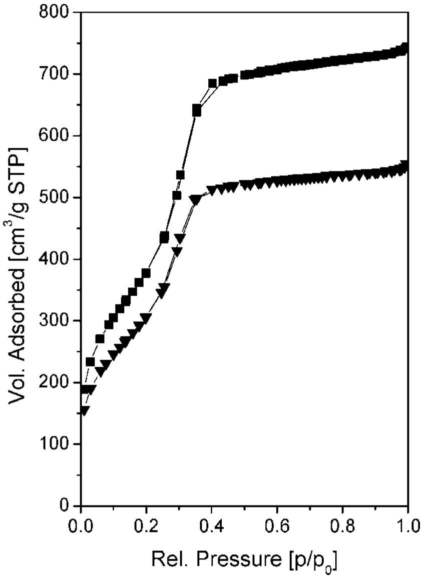

Figure 2. N_2 sorption isotherms for ethanol-extracted SBA-3 (■) and postextracted PEI-SBA-3 (▼).

in the pores of postextracted materials, partial blocking of some of the porosity after PEI functionalization, and, more importantly, remaining water, especially in intrawall pores (see Supporting Information) due to the low sample activation temperatures of 323–333 K used during the outgassing step of the PEI-functionalized materials. This low temperature was used as PEI has been reported to start to decompose at 343 K on flat silica surfaces.[23] Thus, the surface area and pore volume values cannot be directly comparable. However, functionalization of the mesopore walls of such small-pore systems would intuitively be expected to lead to more pronounced effects on the pore volume and pore diameter. This is also seen for surface functionalization of the same SBA-3 material using 5 wt % aminopropylsilane, leading to a pore size decrease of 0.6 nm and a total pore volume decrease of more than 30% (results not shown), and hence the fact that the pore diameter is not affected by PEI functionalization suggests that the primary location of PEI is on the outer surface of the particles. However, it is not possible to fully exclude the presence of some PEI also in the mesopores, especially close to the pore entrances, on the basis of the available data. The presence of PEI on the outer surface of the SBA-3 particles is also seen as a prominent increase in the value of the isoelectric point (IEP) upon PEI functionalization (from about 4.2 to 9.8; see Table 1), as zeta-potential measurements are sensitive mainly to the charging at the outer surface of the particles. The intrinsic pK_a value of the PEI is 10.6, and the IEP should thus be shifted toward higher values due to the presence of positively charged $-NH_3^+$ groups on the outer particle surface. The IEP of native SBA-3 is higher than that expected for amorphous silica, for which values in the range 2–3 are normally observed. The reason for this difference is not known at present, but we note that the absolute value of the zeta-potential of amorphous silica is normally very small in the pH range 1–4, which can lead to fairly different IEP values for similar materials. However, the higher than expected IEP of the SBA-3 substrate does not change the

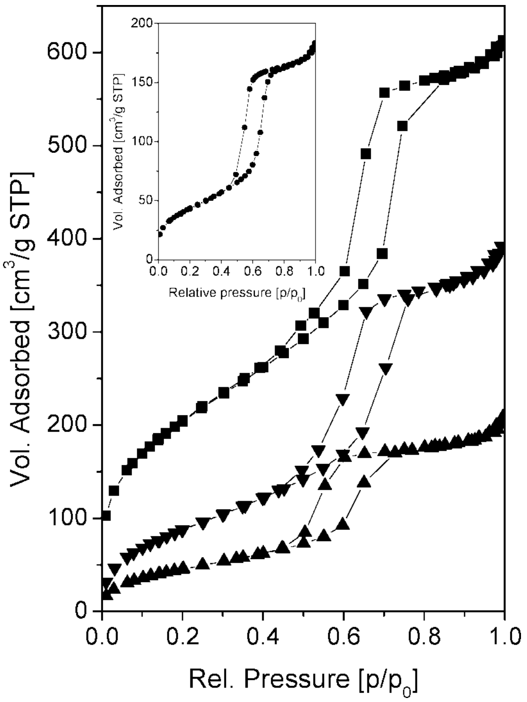

Figure 3. N_2 sorption isotherms of the co-condensed substrate material CC-SBA-15 (▲) which has been PEI-functionalized before (■) and after (▼) extraction with the same amount of PEI. Inset: postextracted succ-PEI-CC-SBA-15.

conclusions made related to the preferential surface functionalization of the outer surface of the SBA-3 particles.

PEI Functionalization of a Co-condensed Amino-SBA-15 Material. The approach described above can also be used for the synthesis of mesoporous materials with functional groups other than silanols present on the mesopore walls. This is demonstrated for amino-functionalized SBA-15 synthesized by co-condensation of TEOS and APTES. The nitrogen sorption isotherm of a co-condensed material prepared with 10% APTES is shown in Figure 3, and the capillary condensation step centered on a relative pressure of 0.7 corresponds to a mean mesopore diameter of about 7 nm. The N_2 sorption isotherm of the amino co-condensed SBA-15 material PEI functionalized before surfactant extraction is also shown in Figure 3. The capillary condensation step occurs within the same relative pressures as for the substrate, indicating that the mesopore diameter does not change upon PEI functionalization. However, it is known that part of the surfactant can be removed from SBA-15 by simple washing procedures due to weak interactions between the silica and the surfactant and why it is naturally possible that some of the pore surface has been PEI functionalized as well, although also in this case a direct comparison of the specific surface area and pore volume values should not be made due to the possibility of remaining water in intrawall porosity of the functionalized materials (see Supporting Information). We therefore also performed a PEI functionalization with the same amount of aziridine of corresponding SBA-15 substrates but where the surfactant had been extracted before the functionalization step. The nitrogen sorption isotherm of a co-condensed amino SBA-15 which has been PEI functionalized after extraction[15] of the surfactant is also shown in Figure 3. PEI functionalization after surfactant extraction leads to a decrease in the mesopore diameter, and the values for the specific surface area, mesopore diameter, and the mesopore volume are both clearly lower for the postfunctionalized material, which gives further support for our claims that the PEI function is indeed preferentially located on the outer surface of the particles if the functionalization step is carried out before surfactant extraction. The IEP of the PEI-functionalized material was 10.4, again in agreement with a high surface concentration of amino groups on the outer surface of the particles. The IEP of the co-condensed SBA-15 was about 8, indicative of the presence of amino groups on the outer surface of the particles, but the clearly lower IEP of the co-condensed material shows that the concentration of amino groups on the outer surface of the co-condensed particles is lower than that on the PEI-functionalized particles, as discussed in much more detail elsewhere.[15] The same concept can be used to prepare similar materials, but with other functional groups on the mesopore walls, if other functional silanes are used for co-condensation, provided the functional groups do not interfere with the PEI growth on the surface.

Table 2. Critical Values for the Material Series Investigating the Relation between the Succinic Acid and PEI Functionalization[a]

sample	IEP	new funct[b] [wt %]	total org [wt %][c]
CC-SBA-15 extracted (substrate)	8.14		4.3
PE-PEI-CC-SBA-15	11.1	27.2	30.3
succ-PE-PEI-CC-SBA-15	7.05	14.2	37.5
PE-succ-PEI-CC-SBA-15	7.64	19.5	41.4
2nd succ-PE-PEI-CC-SBA-15	5.84	24.3	44.9
PE-$^1/_2$PEI-CC-SBA-15	10.1	17.3	20.9
succ-PE-$^1/_2$PEI-CC-SBA-15	5.88	15.3	30.0
PE-succ-$^1/_2$PEI-CC-SBA-15	6.73	17.7	31.9

[a] Code for material names: PE = postextracted; succ = succinylated; PEI = PEI-functionalized; $^1/_2$PEI = half the aziridine amount used as previous; CC = co-condensed amino-SBA-15. The materials are indexed so that the last performed functionalization step appears first, etc. [b] The mass increase recorded for the functionalization step in question from TGA. [c] Total organic content of the material as estimated from TGA.

Succinylation of the PEI-Functionalized Co-condensed Amino-SBA-15 Material. In a further development, carboxylic acid groups were introduced into the PEI layer by treatment of PEI-functionalized materials with succinic anhydride, as described in Scheme 1 in the Experimental Section. Attempts to directly succinylate the co-condensed SBA-15 material did not result in any introduction of carboxylic acid groups, regardless of whether the succinylation was carried out on dried as-synthesized materials with the surfactant still remaining in the pores or whether the succinylation was performed after removal of the surfactant by extraction, as evidenced by the fact that the IEP of the succinylated co-condensed materials remained virtually the same as before succinylation. The N_2 sorption isotherm measured for a succinylated PEI-CC-SBA-15 material is shown in Figure 3 as an inset. Succinylation did not lead to a change the mesopore diameter, which is another indication for the preferential location of PEI on the outer surface of the particles. The *C* value also increased from 30 to 60 upon succinylation, which reflects the introduction of more polar groups onto the surface.

Succinylation of the PEI-functionalized co-condensed material lead to a marked decrease in the IEP from 10.4 to about 6.5 after surfactant extraction. The decrease in the IEP suggests that succinylation has been successful and that carboxylic acid groups are present in the surface layer. This is also readily seen in the thermogravimetric data, as the mass loss recorded for the succinylation step is about 15 wt %. However, the fact that the IEP is well above the pK_a of the carboxylic acid group (pK_a about 4.2) suggests that there are still remaining amino functions in the PEI polymer layer. This is not surprising, as primary, secondary, and tertiary amino groups can be present in the PEI polymer, depending on the location of the amino groups and also on the degree of branching. The different amino groups all have pK_a values close to 11, so these groups will partly neutralize the charge of the introduced carboxylic acid functions. To more closely investigate the relation between the interdependency between the amount of PEI and the amount succinic acid groups added, a new series of materials were prepared. The main characteristics of these materials are summarized in Table 2. The PEI content of the PEI-functionalized materials was about 27 wt % in this case. Another set of materials were prepared using only half the amount of aziridine in the functionalization step, and these materials are indicated by $^1/_2$-PEI in Table 2. Succinylation was performed either before or after extraction. For one PEI material, succinylation was performed both after and before surfactant extraction. The XRD pattern for this material is also shown in Figure 1. This second cycle of succinylation led to a further decrease in the IEP to 5.8 from the initial IEP of 11.1, which suggests that the amount of introduced carboxylic acid functions can be rationally controlled but also suggests that not all amino groups are accessible for succinylation. Furthermore, this observation also suggests that it is the outer, more easily accessible, amino groups of the PEI surface layer which are preferentially succinylated. The C value for the doubly succinylated material ($C = 65$) increased slightly as compared to that of the material just succinylated once (Table 1), again reflecting the increasing polarity of the surface. The total amount of succinylated groups achieved via the repeated succinylation was about 24 wt %.

Adsorption of Salicylic Acid to the Amino Groups of a Succinylated (Acidic−Basic) Material. A convenient means of proving the presence and accessibility of amino groups is the adsorption of salicylic acid (SA), which adsorbs strongly to amino groups in water already at low pH due to its low pK_a value of about 3.0. The silanols in the SBA-15 material start to deprotonate at a pH of about 2, but most silanols do not deprotonate at pHs below 7.[25] The pH-dependent adsorption of SA to the PEI-functionalized SBA-15 which was subsequently succinylated twice is shown in Figure 4 along with the zeta-potential curve of the same material. As a reference material we chose a carboxylic acid-functionalized SBA-15,[26] in which about half of the accessible surface groups that can deprotonate at a pH of 10 are carboxylic acid functions and half are silanols.[25] For the PEI-SBA-15 material succinylated twice a U-shaped SA adsorption curve was obtained, with maximum adsorption around pH 3. The origin of this curve shape is readily explained on the basis of electrostatic considerations, as an increasing fraction of SA remains in the carboxylic acid form below pH 3, and thus the electrostatic driving force for adsorption to the positively charged amino groups is gradually lost. In addition, the ionic strength at low pH also serves to screen the electrostatic attraction between oppositely charged groups. Above a pH of 3, the silanol groups and the carboxylic acid groups will start to deprotonate to an extent which is increasing with pH, and thus the effective surface charge of amino-functionalized materials will be less and less positive until the IEP is reached, which again serves to decrease the electrostatic driving force for adsorption of SA to the materials. Little or no adsorption of SA was observed for the carboxylic acid-functionalized SBA-15 material, which is in line with the absence of positively charged amino groups in this material, and the results confirm that the observed pronounced adsorption of SA to the twice succinylated PEI-SBA-15 material, with a maximum surface excess of about 8 μmol/m^2, indeed can be mainly ascribed to the adsorption of SA to amino groups. The high surface excess values obtained for the succinylated PEI SBA-15 material suggests that the remaining amino groups are accessible to SA adsorption, and the results also confirm that amino groups still remain in the PEI layer, most probably preferentially located in the inner portion of the surface layer, as discussed above.

Variation of the Relative Amount of Surface Layer Groups as a Means of Controlling Particle Effective Charge. The finding that the IEP of succinylated PEI materials can be controlled by the level of succinylation also suggests that the thickness of the PEI layer will have an influence on the IEP after succinylation. The number of remaining amino groups after succinylation should be lower if the PEI layer is thinner, if our assumptions are correct. We therefore carried out the PEI functionalization step using only half of the PEI amount used in the functionalization of CC-SBA-15 described above. The IEP after PEI functionalization was still high, 10.1, but slightly lower than that obtained under the standard conditions for the corresponding material, which was 11. After one succinylation cycle the IEP decreased from 10.1 to 5.9, which can be compared to the corresponding value measured for the SBA-15 material functionalized using the higher PEI concentration, which was slightly above 7. In fact, the IEP of 5.9 corresponds almost exactly to the IEP measured after two cycles of succinylation of the material where double the amount of PEI was used in the functionalization step. We note that the influence of remaining surface silanols on the IEP values should be small in this pH range,[25] which further gives support for the conclusion that the ratio of carboxylic acid to amino groups after succinylation can be controlled both by changing the thickness of the PEI layer by simple adjustment of the PEI-to-silica ratio used in the functionalization step and by varying the degree of succinylation. This makes our approach very flexible, as the thickness of the PEI layer and the amount of remaining amino groups should have a pronounced

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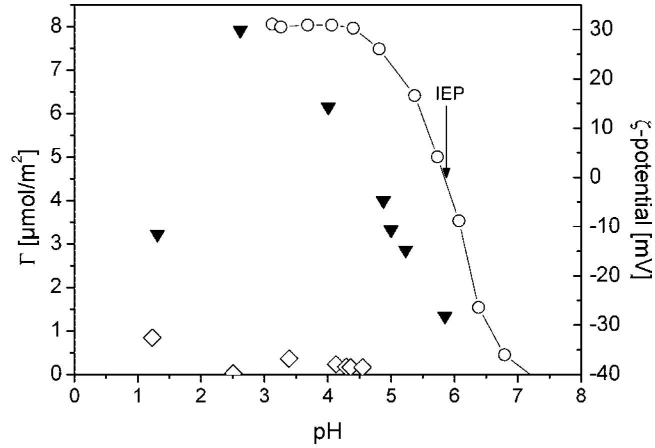

Figure 4. Adsorption of salicylic acid as a function of pH from a 15 mM aqueous solution for the twice succinylated material 2nd succ-PEI-CC-SBA-15 (▼) vs COOH-SBA-15 (◊) as reference, to illustrate the preferential adsorption to amino groups. Also included is the zeta-potential curve for the 2nd succ-PEI-CC-SBA-15 material (○).

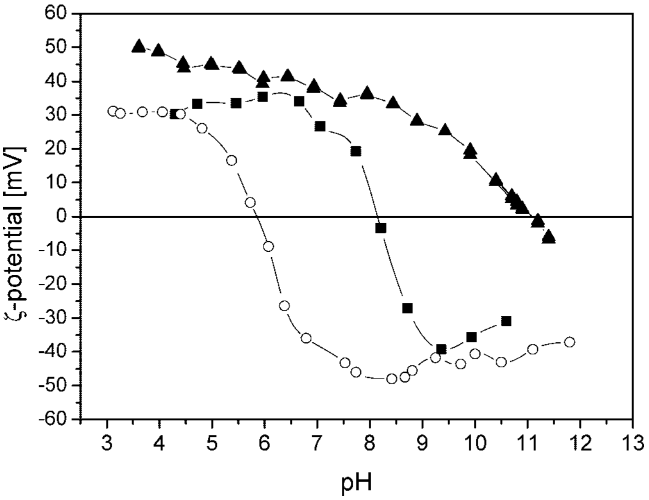

Figure 5. Electrokinetic titration curves for CC-SBA-15 (■), postextracted PEI-CC-SBA-15 (▲), and the twice succinylated 2nd succ-PEI-CC-SBA-15 (○) materials presented in Table 2.

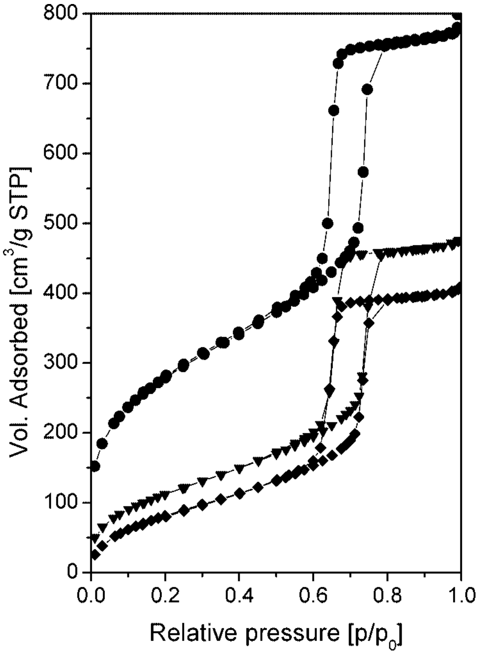

Figure 6. N_2 sorption isotherms of ethanol-extracted SBA-15 (●), postextracted PEI-SBA-15 (▼), and HMDS-treated postextracted PEI-SBA-15 (♦).

influence on the molecular gate efficiency of the surface layer, and the amount of carboxylic acid groups is important for fine-tuning of the IEP for a given application, which we postulate will be a highly useful approach for targeted delivery of drugs into specific tissues, as the internal pH is 6.8 in tumor and inflammatory tissues,[27] 5.5–6 in endosomes,[28] and 4.5–5.0 in lysosomes.[28] Furthermore, the possibility of tuning the surface concentration of carboxylic acid and amino groups also provides further flexibility for tuning the surface concentration of other surface functions that are added in subsequent steps using standard amino- or carboxylic acid-based linking chemistry.

Implications of the Effective Charge Determined by Electrokinetic Measurements. At this point we will return to discuss the electrokinetic data of the functionalized native and succinylated PEI-functionalized SBA-15 materials in more detail, as it is well-known that an absolute zeta-potential of about 30 mV is needed in order to ensure a good electrostatic stabilization of particles in solution. The zeta-potential vs pH curves measured for the twice succinylated PEI-functionalized SBA-15 material discussed above, before and after succinylation, as well as for the co-condensed amino SBA-15 material used as substrate are shown in Figure 5. In the pH range of highest biological interest, close to neutral, both the PEI-functionalized and succinylated PEI-functionalized SBA-15 materials fulfill the criteria of electrostatic stabilization, but the surfaces are oppositely charged in the two cases. The co-condensed amino SBA-15 material, which has an IEP of about 8, does not fulfill this criteria in the pH range of interest, which is why it can safely be assumed that the co-condensed SBA-15 not surface functionalized with PEI will aggregate strongly under these pH conditions. Thus, selective surface functionalization of mesoporous silica by PEI is a promising method also for enhancing dispersion stability under close to neutral conditions. However, we want to stress that the absolute zeta-potential is also influenced by the electrolyte concentration in solution, and electrostatic stabilization may not prove to be very successful under highly saline conditions. However, the PEI surface layer should also offer a means for steric stabilization, which is why the positive effect of PEI on dispersion stability may still be effective also under such conditions.

Preparation of a SBA-15 Material Exhibiting Hydrophobic−Hydrophilic Properties. So far we have described functionalization of mesoporous silica with PEI preferentially located on the outer surface of mesoporous silica particles. Such particles have different Brønsted properties on the outside as compared to the inside surfaces, as PEI exhibits Brønsted basicity and the silanols on the mesopore walls

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exhibit Brønsted acidity. However, it would be useful to be able to also alter the surface function on the pore walls as well independently from the surface functions on the outer surface of the particles. Especially interesting could be particles being hydrophilic on the outer surface but containing a hydrophobic internal mesopore system. This would allow incorporation of hydrophobic molecules not having functional groups into the confined space of the mesopores, still maintaining a good dispersibility of the particles in aqueous solutions. Such particles would thus carry some similarities with surfactant micelles and surfactant-stabilized emulsion droplets. We therefore functionalized purely siliceous SBA-15 with PEI before surfactant extraction and subsequently exposed the extracted PEI-SBA-15 material to hexamethyldisilazane (HMDS) vapor. HMDS treatment is a common hydrophobization procedure applied to mesoporous silica in order to increase its hydrothermal stability[29] by capping residual silanols.[30] The presence of PEI on the outer surface of the particles was clearly seen in the corresponding IEPs, which increased from about 3 to 9.6 upon PEI functionalization, a value which remained unaffected by HMDS treatment. We note that attempts to grow PEI from the outer surface of inherently hydrophobic mesoporous silica particles, like phenyl-SBA-1 synthesized by co-condensation,[31] did not prove to be successful. The nitrogen sorption isotherms measured for the PEI-SBA-15 and HMDS functionalized PEI-SBA-15 are shown in Figure 6. The sharp capillary condensation step occurs at similar relative pressures for all materials, corresponding to a mesopore diameter of 7.9 nm, despite a total loading of as much as 28 wt % PEI, suggesting that the PEI is preferentially located on the outer surface of the SBA-15 particles. However, we note that the NLDFT kernel used for analysis of the mesopore diameters is developed for silica, and this could lead to slight errors in the pore diameter values for the hydrophobized SBA-15 material. Again, the specific BET surface area and the specific pore volume of PEI-functionalized SBA-15 is lower than that of the parent SBA-15 material, partly due to the increase in sample mass upon PEI incorporation, but also here the decrease in the specific surface area and pore volume is higher than expected for PEI exclusively located on the outer surface of the particles. The *C* value, decreased from 107 to 42 after PEI functionalization, which reflects the decreasing mean polarity of the surface upon introducing organic PEI functions on the outer particle surface still having the silanols being exposed on the walls of the mesopores. The *C* value decreased further to 24 for the HMDS-functionalized PEI-SBA-15 material, in good agreement with the expected lower polarity of the silica pore walls upon silanization, as well as compared to a HMDS-functionalized calcined SBA-15 which has a *C* value of 28 (see Table 1). For completion, the XRD patterns measured for the HMDS-functionalized PEI-SBA-15 material is shown in Figure 1, confirming the preservation of the long-range order in the SBA-15 material throughout the functionalization process, as evidenced by the three clearly resolvable high-order reflections. The HMDS−PEI-SBA-15 particles could be easily dispersed in water (pH = 7), as confirmed by turbidity measurements (see Supporting Information), while it was impossible to disperse corresponding SBA-15 particles functionalized by HMDS but without the PEI layer on the outer surface.

Conclusions

A highly flexible method for selective functionalization of mesoporous silica has been presented, where the key step is the preferential growth of hyperbranched poly(ethylene imine), PEI, on the outer surface of the material. This can be achieved by growing the polymer before the surfactant used as structure-directing agent is extracted from the mesopores of the silica. The thickness of the PEI layer can be controlled by adjusting the PEI/silica ratio during the functionalization step. Furthermore, carboxylic acid functionalities can be introduced preferentially to the outer part of the PEI layer by applying succinylation, and the relative concentration of carboxylic acid to amino groups in the PEI layer can be controlled by variation of (a) the number of succinylation cycles, (b) the PEI layer thickness, and (c) the succinic anhydride concentration used in the succinylation step. This makes it possible to fine-tune the surface charge of the particles for a given application independently from the thickness of the surface layer, which is expected to offer advantages in applications where further surface functionalization is needed, in combination with molecular gate properties and/or a high dispersion stability, which all are important parameters in drug delivery applications. The surface functions are accessible and covalently linked to the particle, which is important in order to ensure that targeting functions do not detach from the particle, which is not necessarily the case when the surface layer is only adsorbed to the particle surface.

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Supporting Information Available: NLDFT pore size distributions for CC-SBA-15, postextracted PEI-CC-SBA-15, PEI-CC-SBA-15, extracted SBA-3, and postextracted PEI-SBA-3; turbidity transmission profile after sonication. This material is available free of charge via the Internet at http://pubs.acs.org.

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