Non-Peptide Polymeric Silicatein α Mimic for Neutral pH Catalysis in the Formation of Silica

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ABSTRACT: We have synthesized a catalytically active polymer inspired by the naturally occurring protein silicatein α and have shown it to catalyze the formation of silica from tetraethoxysilane under near-neutral pH and ambient temperatures. We based the composition of the polymer on the functionalities found in silicatein α, specifically those essential components of the catalytically active site for the hydrolysis of silicon alkoxides. Our bioinspired polymer is a block copolymer of poly(2-vinylpyridine-*b*-1,2-butadiene), functionalized by the addition of hydroxyl groups via hydroboration chemistry. The catalytic action of our polymer on tetraethoxysilane at neutral pH and ambient temperature conditions has been confirmed using a modified molybdic acid assay method, thermogravimetric analysis, and Fourier transform infrared spectroscopy. The structure of the resulting gel is investigated by scanning electron microscopy and solid-state nuclear magnetic resonance. The microscopic features of the material formed resemble that of gels formed by the acid-catalyzed hydrolysis of tetraethoxysilane.

Introduction

In the absence of catalytic agents, the hydrolysis and condensation of tetraethoxysilane (TEOS) are slow processes in neutral or near-neutral solutions,[1,2] useful for following the progress of silicification but limiting for practical applications.[3] High- or low-pH solutions are typically used to speed the rates of reaction for the hydrolysis or condensation of the silica precursor to form the condensed phase.[4] For example, the well-known Stöber process[5] for the synthesis of colloidal silica particles uses solutions of high ammonia content and high pH (>10). In contrast, natural processes exist for templating silica at neutral pH and under ambient temperature, combining catalysis and structure-directing in the formation of an inorganic phase.[6-8] While some synthetic templates are stable at low pH,[9-11] these are limited in the types of structures formed.[12,13] However, increasing the rates of hydrolysis or condensation under purely synthetic conditions by adding acid or base catalyst, or just by heating the solution, may adversely affect included materials such as organic templates or functionalizing agents by introducing unwanted reactions or phase changes.[9]

Patterned ceramic nanostructures can be synthesized using synthetic organic molecules—surfactants and block copolymers—as templates for the nucleation and growth of the inorganic phase.[14] There is extensive literature on the silicification of these templates using TEOS as the source of silicon oxide but usually requiring solutions of high or low pH, well away from conditions of physiological or near-neutral pH.[14,15] The neutral template of Tanev and Pinnavaia,[16] although not strictly speaking a neutral pH process, demonstrated that neutral surfactants (S) could combine with neutral inorganic precursors (I) to form structured nanoporous matrices. But in typical synthetic systems, acid or base provides the primary catalytic function with the
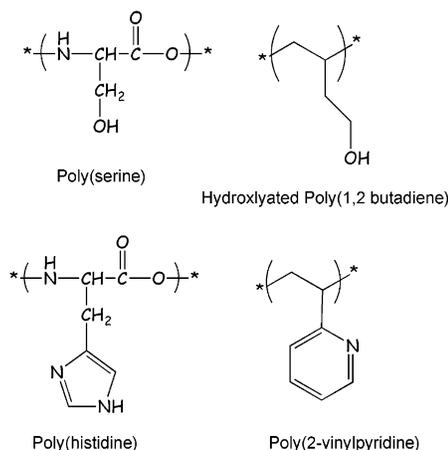

Figure 1. Comparison of the natural functional groups and the synthetic functional groups. The structures on the left are the amino acids found to be active in silicatein α hydrolysis of TEOS. The structures directly to their right are the functional groups incorporated into our fully synthetic material.

organic acting as the template. Since biomolecules exist that satisfy both roles,[17-19] our goal is to develop purely synthetic analogues to these natural materials. In this report, we focus on the synthesis of a polymer that mimics the catalytic function of a recently discovered natural catalyst,[17] silicatein α.

The protein silicatein α has been previously reported[12,17-19] to catalyze the production of poly(silicate) from TEOS precursor at neutral pH under ambient temperatures. This protein was isolated from the marine sponge *Tethya aurantia*, a sponge whose structure is defined by silica needles that constitute up to 75% of its dry weight.[17] Occluded in these needles are protein filaments that direct the growth of the needles while catalyzing mineralization. About 70% of this organic component is the protein silicatein α. In this phase of our work, the goal is to emulate the natural system by building a non-peptide-based synthetic analogue that can be used to produce silica and may be extended to other oxides such as TiO₂.[20]

By employing site-directed mutagenesis,[17] the amino acids critical to the hydrolysis of TEOS were determined. Figure 1 illustrates the chemical structures of the hydroxyl of serine-26

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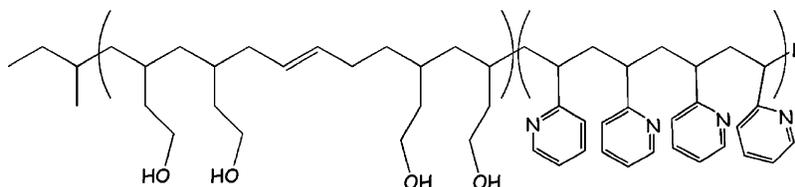
Figure 2. Structure of the synthetic diblock copolymer made in this study. The first block is polybutadiene that has been modified through hydroboration chemistry. The second block is poly(2-vinylpyridine).

and the imidazole of histidine-165 that were shown to play key roles in the hydrolysis of TEOS.[19] These functionalities were proposed as participants in the formation of a transitory pentavalent silicon species stabilized through a donor bond to the imidazole nitrogen. This mechanism for the hydrolysis of TEOS is analogous to the mechanism of peptide bond hydrolysis by the homologous protease cathepsin L.[21] On the basis of this information, our initial goal is to synthesize a polymer incorporating both the hydroxyl functionality and a nitrogen-containing heterocycle. Reported here is the synthesis of the catalytic analogue and proving its catalytic activity in the formation of silica from a soluble precursor. The polymer we have synthesized, poly(hydroxylated butadiene-*b*-2-vinylpyridine), incorporates the monomers illustrated in Figure 1.

Mimicking silicatein α with synthetic polypeptides has been demonstrated previously.[22] In that study, block copolypeptides were synthesized by a ring-opening polymerization method using cyclic amino acid anhydrides as monomers. Homo- and block-proteins were made and tested for catalytic function in the hydrolysis and subsequent condensation of TEOS. Those results proved that the tertiary structure of the protein was not critical to its catalytic function and provided further evidence as to which amino acids, and thus which functional groups, were critical to the protein's catalytic function. It was shown that the hydroxyl group of serine was active in catalysis. However, because of difficulties with protecting chemistries, the histidine residue was not incorporated into the synthetic peptides; instead, its hydrogen-bonding amino functionality was provided by an amino acid with a primary amine side chain.

Other studies[25-29] have addressed the use of proteins to template the condensation of silicic acid and its oligomers to form silica. But no catalytic mechanism such as that proposed for our silicatein α mimic was suggested as no hydrolysis of the precursor was required. An example is the work of Mizutani et al. that showed poly(allylamine), poly(lysine), and poly-(arginine) accelerate the precipitation of silica from silicic acid, $Si(OH)_4(aq)$.[23,24] In another study using silicic acid as the silicon oxide source, poly(lysine) and poly(arginine) polymers were shown to effect the condensation of silica.[25,26] In these studies, the proteins appear to serve as templates for condensation. Electrostatic interactions between the positively charged amino groups and the charged silicon oxyhydroxide complexes led to rapid precipitation. A recent example that used TEOS rather than silicic acid incorporated a cationic synthetic polymer, poly-(allylamine) hydrochloride, in an attempt to direct the formation of poly(silicate) at ambient conditions.[27] It was found that prehydrolysis of TEOS using HCl(aq) was necessary before the polymer could be used to form poly(silicate) and that the hydrolysis time was critical to determining the resulting material. This is in marked contrast to our system in which TEOS is catalytically hydrolyzed at near-neutral pH. Other proteins and polymers have been studied.[28,29] These systems involved the use of proteins with either prehydrolyzed monomer or with the more easily hydrolyzed monomer tetramethoxysilane (TMOS) rather than the less reactive TEOS used in our work. Bioinspired
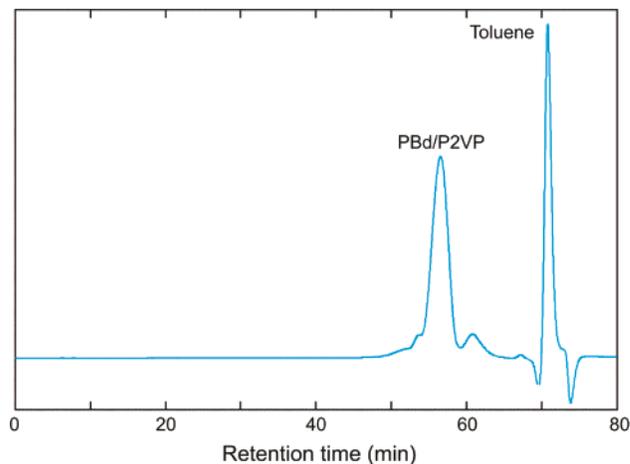

Figure 3. GPC trace of poly(1,2-butadiene-*b*-2-vinylpyridine), PBd/P2VP. The large peak to the right is toluene. This figure indicates that polymer synthesized is a diblock copolymer rather than two distinct homopolymers. The small shoulder to the left is likely coupled block copolymer while the small peak on the right is unreacted polybutadiene homopolymer.

small-molecule catalysts have also recently been developed using these principles, although these small molecules do not exhibit any templating activity.[30-32] In contrast to monomers and nanoparticle-supported self-assembled monolayers, our system provides an avenue for mimicking the structure-directing property of the protein, relying on the characteristic microphase separation known for systems of block copolymers.[33]

Methods for controlling the structure of silica also have been reported previously. As one example of the extensive literature on surfactant-directed structure formation, the neutral templating route involves the self-assembly of uncharged primary amines with neutral TEOS to form nanoporous silica with small pore diameters.[16] Also, the use of polymers as structure directing agents has been studied extensively.[9,34-37] For example, poly-(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (Pluronics) is a popular agent.[21] These systems have been very successful in producing larger pore size nanoporous silica systems but most require low pH to hydrolyze the siloxane prior to assembly. A truly bioinspired system avoids extreme pH, and we show in the present study that our synthetic polymer (Figure 2) fits into this category.

Results and Discussion

Polymer Synthesis. The heart of our effort to mimic the enzymatic activity of the natural protein is the incorporation of functionalities found to be critical to the natural process. In Figure 3 we show the gel permeation chromatography (GPC) trace of the block copolymer we have synthesized. The single peak in the GPC trace indicates that polymerization produces monomodal polymer, as depicted in Figure 2. The shoulder on the left of the block copolymer peak indicates some linking reactions which likely occurred during the termination of the

polymerization. After hydroxylation, the polymer is soluble in a 50:50 ethanol:water solution (by volume).

The polymer's solubility in what is a nonsolvent of the parent material and our observation that it is not soluble in common hydrocarbon solvents such as toluene or cyclohexane are indications that the polymer has been hydroxylated and is now more hydrophilic than the parent material. Proton nuclear magnetic resonance (NMR) of the final product in deuterated chloroform shows the vinyl protons found in the parent material have disappeared, as would be expected after extensive hydroboration. For the unfunctionalized material, shifts were assigned as $[-CH_2-CH=CH-CH_2-]$ (2 protons) at $\delta = 5.45$ ppm and $[-CH_2-CH(CH=CH_2)-CH_2-]$ (2 protons) at $\delta = 4.92$ ppm for the vinyl protons in the poly(butadiene) block. Because of the promoter used during the polymerization, the polymer was enriched in 1,2 addition and contained ~63% 1,2 addition. After hydroboration, the 1,2 content was reduced to ~28%, indicating hydroxylation of most, though not all, of the pendent double bonds.

Degree of Poly(silicate) Condensation. The molybdic acid assay of the reaction product of polymer and TEOS indicates the amount of solid poly(silicate) formed. In an effort to more directly compare our material with the natural protein, we used concentrations and reaction times similar to those used in assays of the natural material.[18] We thus present our finding in units of μmol h^{-1}. It is important to note that the molybdic acid assay will not accurately measure the total amount of silicate but only that of the silicate available to form the silicomolybdate complex. Only straight chains of very short length will react to form the colored complex. Poly(silicate) chains as short as three or four monomer units may be too long to react by this method.[38–40] Completely digesting the precipitate in a sodium hydroxide solution largely overcomes this limitation by dissolving the precipitate through complete hydroxylation of the silicon atoms in the condensed silicate, creating monomeric silicic acid molecules. Using 3.0 mg of polymer (0.6 mL of a 5.0 mg/mL solution), we found that our polymer catalyzes the formation of poly(silicate) at the rate of 0.11 μmol h^{-1} while the rate of a control reaction containing no polymer is less than 0.01 μmol h^{-1}. In general, we observed that adding TEOS to a polymer-containing solution immediately resulted in a cloudy suspension. In contrast, adding TEOS to solutions without the polymer present did not immediately cause clouding.

The rate of silica formation in our system was also within an order of magnitude of the rates reported for poly(amino acids).[22] For this comparison we used the same concentration of polymer as was used for the poly(amino acids). In both cases, the amount of TEOS used was in large excess to ensure that the polymer was the limiting reagent. In addition, the pH of the system was monitored and found to range between 6.6 and 6.8 without the use of a buffering agent. A direct comparison of rates with the natural silicatein α was not possible as absolute rates were not reported for the natural material; however, the amounts of silica formed relative to a control were reported. Comparing the amounts of silica polymerized by 0.3 mg of the native protein with the amount polymerized in a control reaction revealed that silicatein α polymerized 21 times more poly(silicate) than did the control within 15 min. An equivalent amount of the denatured version of the native protein polymerized only 2.4 times as much as the control within 15 min. Our synthetic polymer formed 11 times more silica than did a comparable control reaction over a reaction duration of 1 h. The controls used for our polymer included (i) no polymer present in the system and (ii) using homo-poly(vinyl alcohol). The use of a random copolymer was not investigated, as anionic polymerization does not produce a truly random copolymer of 2-vi-
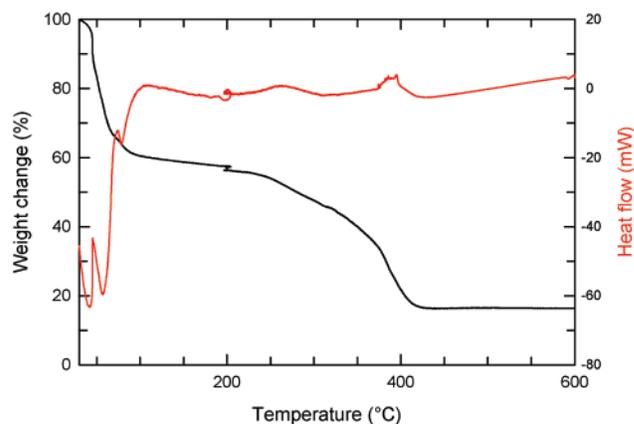

Figure 4. TGA of solid precipitate formed by the reaction of TEOS with the bioinspired PBd-OH/P2-VP material. Initial heating to 200 °C removes volatiles, leaving a solid mixture of inorganic and organic materials (60% of the original sample weight). Further heating to 600 °C removes the organic component and results in an inorganic solid (15% of the original sample weight), indicating that 25% of the dried organic/inorganic mixture is silica.

nylpyridine and butadiene under the conditions used in our synthesis. Additionally, poly(2-vinylpyridine-*b*-butadiene) did not show catalytic activity.

We propose that these results can be explained by considering the structures of the three polymers: the native protein with an intact tertiary structure, the denatured protein with no tertiary structure, and the synthetic material which has no tertiary structure but which does have large numbers of alcohol and pyridine functional groups along its backbone. In the native protein, the serine and histidine residues proposed to be critical to the hydrolysis reaction are held fairly rigid. They are in the proper orientation for the catalytic reaction. In the denatured material, the residues are no longer fixed in the required orientation and so catalyze hydrolysis of the TEOS only when random chain fluctuations result in the formation of temporary catalytic sites. The synthetic polymer also lacks the tertiary structure needed to hold the required functional groups in place. This problem is partially overcome by the large numbers of functional groups present, increasing the likelihood that at any time two functional groups will be in the proper orientation and catalyze hydrolysis.

In order to determine the amount silica that forms, we used thermal gravimetric analysis (TGA). The weight loss data up to 600 °C, shown in Figure 4, indicates that mixing dissolved polymer with TEOS to form a two-phase system yielded ~25% of the total weight of the dried precipitate as poly(silicate). The TGA trace is very similar to that for alcogels formed through the acid-catalyzed hydrolysis and condensation of TEOS,[41] with three stages of weight loss. In the first stage, heating the sample to 100 °C results in the loss of entrained solvent and other volatile components. Above 100 °C the material was dry but contained organic and inorganic components. In the second stage, between 100 and ~425 °C, weight loss was primarily due to the pyrolysis of the organic phase and the possible removal of low molecular weight siloxanes. The third and final stage, above 425 °C, exhibited little or no weight loss, and the final material was completely inorganic. The weight fraction of the inorganic was determined by the ratio of the final weight of the inorganic solid (~15% in Figure 4) to the combined dried weights of the organic and inorganic solid (~60% in Figure 4).

However, TGA can underestimate the amount of silica present if the silicon alkoxides are not fully condensed and if low
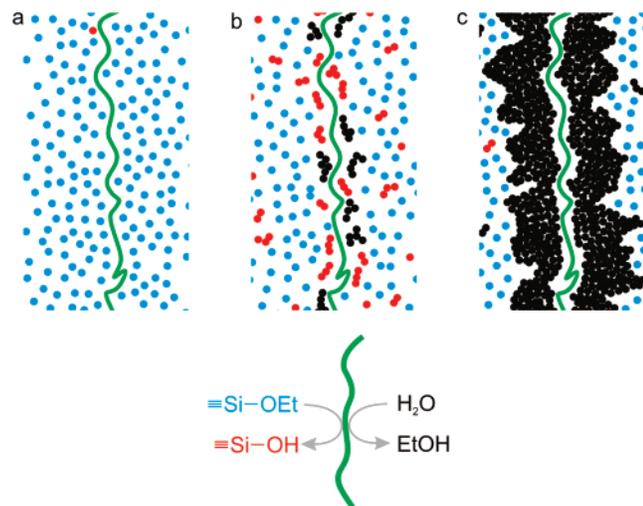
Figure 5. A simplified schematic of the hydrolysis of TEOS to form a barrier surrounding a polymer strand. In this idealized view, the TEOS molecules (blue) are partially hydrolyzed (red) by action of the polymer (green line). The hydrolyzed molecules condense into oligomers as they diffuse away from the polymer. As the local density of the oligomers increases, they aggregate and cross-link to form condensed silica (black). Eventually, the density of the condensate around the polymer prevents further interaction between the catalyst (polymer) and unhydrolyzed ethoxysilanes.

molecular weight siloxanes are removed by washing and/or heat treatment.[41,42] The degree of condensation and the extent of cross-linking needed to incorporate these low molecular weight components into precipitated silica can be improved by lower temperature (<100 °C) heat treatment. We found that heating the precipitate at 90 °C for 3 days to remove solvent and improve the degree of condensation prior to heating the samples to 600 °C did not markedly change the solid/polymer ratio.

The amount of silica formed by this system per unit of polymer is likely limited. As with the biological systems, the polymer is expected to act as a template for silica growth as well as a catalyst for its formation. A growing silica layer can then be expected to increasingly block access to the polymer as silica condenses around it (Figure 5). But the polymer catalyzes the hydrolysis of TEOS, so in the brief interval expected before condensation the hydrolyzed material can diffuse a short distance away from the polymer. Thus, the amount of silica formed is greater than would be expected if only a single layer of poly(silicate) could be formed on the polymer surface. Eventually, a layer of solid material does form around the polymer and stops further catalysis.

This ultimate loss of catalytic function may be exacerbated by the expected structure of the polymer in solution. The more hydrophobic poly(2-vinylpyridine) block is likely to be in the center of a micellar structure formed in the hydrophilic solvent, reducing the number of exposed catalytic sites on the polymer. Changing the volume fraction of the polymer and forming a structure with a higher surface-to-volume ratio should then increase the amount of silica formed per unit of polymer. Studies based on chemical vapor deposition (CVD) suggest another mechanism for the formation of higher-than-expected amounts of silica:[43] that hydrolyzed siloxanes can, in turn, catalyze the hydrolysis of other siloxanes. In this situation, the partially hydrolyzed TEOS acts as a seed for the deposition of additional silica and larger yields could be expected.

Morphology of Silica Product. Scanning electron microscopy (SEM) images of the solid precipitate resulting from the reaction of TEOS with different catalysts are shown in Figure 6. The product of our polymer-catalyzed system resembles that of an acid-catalyzed system in overall appearance,[41] although our polymer-catalyzed reaction was carried out under neutral conditions. This is to be expected if the TEOS is hydrolyzed by the same mechanism proposed for the formation of silica using silicatein α. In that mechanism the rate-determining step in the overall reaction was reported to be the hydrolysis of the TEOS.[18,19] The rate of silica formation from TEOS with acid catalysis is also limited by the rate of hydrolysis of the TEOS, which is then followed by rapid condensation.[1,12] This process results in a denser, smoother surfaced material as poly(silicate) chains grow and cross-link through condensation to form a dense body.[44] In basic solutions, the slower rate of TEOS hydrolysis encourages the formation of particles which then form aggregates,[5] resulting in a material that appears to be composed of small particles (Figure 6). Thus, simply comparing SEM images suggests that the mechanism of formation of condensed silica from TEOS in the natural system is the same as in our synthetic one.

As can be seen in the SEM images of Figure 6, the structure of the silica formed by our polymer, like that of the simply acid- and base-catalyzed materials, does not exhibit any ordered structure. Our interest in this study was to demonstrate that synthetic polymers could be used to increase the rate of formation for silica from near-neutral solutions. We did not attempt to develop the structure-directing properties of the block copolymers, although such molecules have been used, in conditions of low pH, as structure-directing agents in the formation of large pore nanoporous silica.[35,45] Unlike the approach of Tanev and Pinnavaia and many others,[14,16] our system does not rely on self-assembly. In this we cannot claim to approach the full potential of the silicatein α molecule and regard our results to date as a first step in developing a true analogue to the natural protein.

Nuclear Magnetic Resonance. The ^{29}Si MAS NMR spectrum of the as-polymerized silica is shown in Figure 7. Because of the nature of this NMR experiment, the bulk (internal) silicons
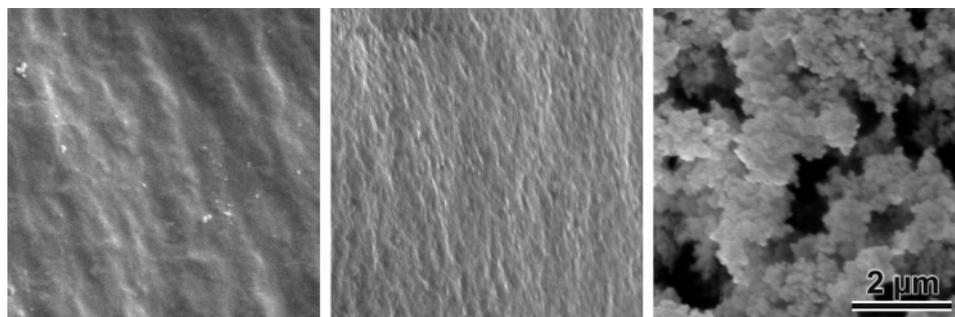

Figure 6. SEM images of solid material from TEOS hydrolysis and condensation using acid (left), polymer (middle), and base (right) catalysts. The surface of the polymer-catalyzed silica resembles the smooth surface of the acid-catalyzed silica, suggesting that the polymer and acid-catalyzed systems have a similar mechanism. Since acid catalyst is known to favor hydrolysis over condensation, this similarity implies that the polymer also favors hydrolysis of the alkoxide. Rapid condensation, seen in the base-catalyzed material (right), results in a particulate structure with larger voids.
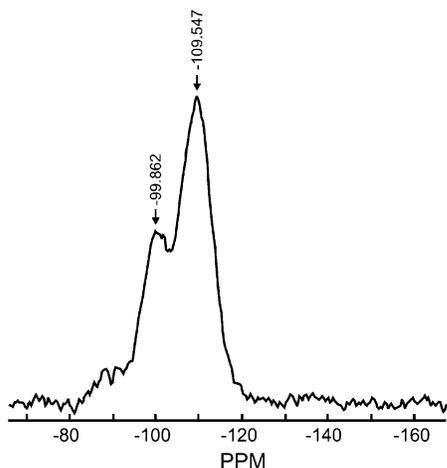
Figure 7. ^{29}Si MAS NMR of the solid poly(silicate) formed by the bioinspired block copolymer and TEOS. The Q_4 to Q_3 ratio is approximately 2:1 with no Q_2 or Q_1 peaks visible.

are fully represented in the spectrum. The spectrum is dominated by the ^{29}Si resonance peak of the siloxanes, **Si**(O—Si)$_4$ or Q_4 sites, present at 109.7 ppm.[46] In addition, there is another resonance peak at 99 ppm, which can be assigned to the single silanols, (Si—O)$_3$**Si**—OH, known as Q_3 sites. Similar results have been reported in studies of fumed silica.[47] Both silicon resonance peaks are broad, typical of amorphous silica which has fluctuations of local arrangement of the Si—O—Si involving bond lengths and bond angles between adjacent Q_4 tetrahedra. Deconvolution of the resonance peaks provides a quantitative estimate of the Q_4/Q_3 ratio that permits us to evaluate the completeness of the polymerization process. The intrinsic mechanical strength of a silica matrix is related to the degree of condensation within the silicon oxide network, so higher condensation implies more cross-linking between silicon centers through oxygen bridges.[4,48]

The ^{29}Si MAS spectrum shown in Figure 7 provides a Q_4/Q_3 of 1.94, using direct integration. Deconvolution of the resonance peaks provides, more accurately, the Q_4/Q_3 ratio, whose quantity indicates the efficiency of the polymerization process. The corresponding computer simulated spectrum (100% Lorentzian, using Varian NMR Software VNMR 6.1C) displaying the sum of the individual contributions from each silicon site produces a Q_4/Q_3 ratio of 2.04, ~5% larger than the direct integration of the spectrum in Figure 7. Regardless of the integration process,

only Q_4 and Q_3 peaks are apparent in the ^{29}Si spectrum, where the Q_2 geminal silanols (at ~−89 ppm) can be considered, if they exist, to be hidden by the noise in the spectral trace.

Kinetics of TEOS Hydrolysis. The hydrolysis of the TEOS is accompanied by the generation of ethanol, typically formulated as[1]

$$n\mathrm{Si(OC_2H_5)_4} + 4n\mathrm{H_2O} \rightarrow n\mathrm{Si(OH)_4} + 4n\mathrm{C_2H_5OH} \quad (1)$$

Condensation of the hydrolyzed TEOS to form silica follows, with the release of water:

$$n\mathrm{Si(OH)_4} \rightarrow (\mathrm{SiO_2})_n + 2n\mathrm{H_2O} \quad (2)$$
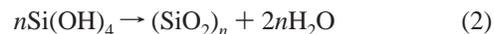
Attenuated total reflectance (ATR) Fourier transformed infrared (FTIR) spectroscopic analysis was used to measure the increase in ethanol content in ethanol/water/TEOS solutions as the TEOS hydrolyzed, providing a simple method for comparing the qualitative rates of hydrolysis among the TEOS-containing solutions at different pH values and in the presence of organic agents. The ethanol absorbance band ~880 cm^{-1} was chosen as the indicator of ethanol content in the liquid phase covering the ZnSe reflectance crystal.[49,50] At this frequency there were no other absorption bands in the studied systems that substantively interfered with the determination of the absolute absorbance; it was required only to establish a simple baseline between 920 and 847 cm^{-1} to measure the relative change in the amount of ethanol over time.[49] This procedure was found to be quite robust and sensitive to changes in ethanol concentration. As care was taken to ensure that consistent amounts of TEOS, water, and ethanol were in used in all solutions, ratioing the absorbance measured at time t to that measured at t_0, the beginning of the experiment, was sufficient to chart the changes in ethanol content with time and visually present the rate of hydrolysis.

In Figure 8 we show representative changes in the normalized ethanol content, providing a comparison of the relative rates of hydrolysis for the different solutions. The cetyltrimethylammonium chloride (CTAC) cationic surfactant can be used to form hexagonal arrays of cylindrical micelles at low pH (≤ 2) and has been observed to speed the formation of silica from TEOS in solution.[51] The combined effect of low pH and surfactant provides an effective catalyst for hydrolysis of the TEOS, especially obvious when compared to the relatively slow initial rate of hydrolysis when using acid alone at comparable pH.
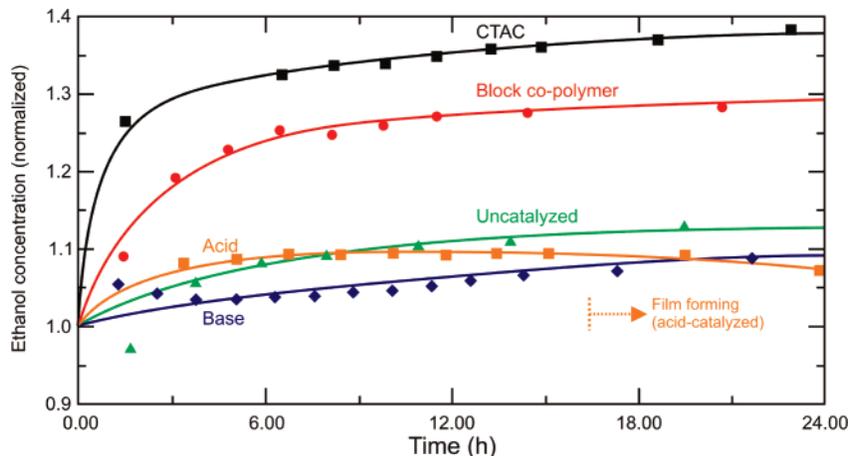

Figure 8. Ethanol produced as a byproduct of TEOS hydrolysis used as a marker for the rate of hydrolysis in different solution systems. The CTAC-catalyzed system (pH ~2) exhibited the fastest rate of ethanol production when TEOS was introduced into the solution. Our synthetic polymer exhibited the next-highest rate of ethanol production under near-neutral conditions (pH slightly above 6). Solutions that lacked an organic agent exhibited comparatively little hydrolysis within the first 24 h of adding TEOS to the respective solutions.

(Here we are not concerned with the structure-directing properties of the CTAC, merely its effect on the rate of hydrolysis of TEOS.) In contrast, our copolymer catalyzes the hydrolysis of TEOS at an almost equivalent rate, but at near-neutral pH (>6). When contrasted to the relative rates of hydrolysis observed in acidic, basic, and neutral solutions, it is obvious that the copolymer is an effective agent in encouraging TEOS hydrolysis.

Above, we remarked on the similarity in appearance between the solid product taken from the acidic solutions and solutions containing our copolymer. There is another marked similarity between the two systems: in these two solutions a film was observed to form at the ZnSe ATR crystal/solution interface. The CTAC system exhibited film formation at the air/water interface, a feature that did not markedly interfere with the spectroscopic measurements on the solutions even beyond 24 h. However, in both acid- and copolymer-containing solutions, the growth of the film on the crystal impeded measurements on the solution after 24 h; as a result, the apparent ethanol content is seen to decrease beyond 24 h. A plateau is observed for the copolymer in Figure 8; in the acid-catalyzed solution there is an obvious decrease in the measured absorbance of ethanol after ~14 h. None of the other solution types exhibited film formation on the crystal. In future measurements this difficulty may be avoided using a flow-through ATR cell. (It should be also noted that the ZnSe ATR crystal can be etched by caustic solution, usually thought to require fairly short exposure times. Other, more resistant crystal materials may be used to circumvent this potential difficulty. We found that the crystal used in our ATR was robust enough to be used for fairly long-term measurements, but we have not fully explored the mechanisms that might explain the formation of films on the crystal surface.)

Conclusions

We have demonstrated the ability of a purely synthetic, non-peptide copolymer to catalyze the hydrolysis of TEOS, leading to the formation of silica by mimicking the functionalities of a natural protein. The hydroxyl functionality of serine-26 and the imidazole functionality of histidine-165, shown to be critical for the catalytic function of the natural protein, were incorporated into our polymer. To our knowledge this is the first example of silica produced using a bioinspired, non-peptide polymer-based catalytic system. Molybdic acid assays show the extent of catalysis to be comparable to peptide-based systems. TGA shows the precipitated material to contain ~25% silica. This may prove to be the upper limit as the reactive sites along the polymer chain are shielded from further reaction by condensed silica. SEM analysis reveals a product structure similar to that of an acid-catalyzed system although the reaction was performed at near-neutral pH. This is consistent with the mechanism of the condensation involving rate-determining hydrolysis as is also proposed for the natural system. Additionally, we find that the extent of polymerization is quite high, with Q_4 values near 75% and Q_3 at 25% as determined by ^{29}Si NMR. Finally, we find that the hydrolysis kinetics of our system appear to be similar to that of a surfactant-catalyzed solution at pH 2.

The ability to form silica in ambient conditions is expected to lead to applications not previously possible using conventional methods. These include environmentally friendly production methods, inclusion of catalysts in nanostructured ceramics, and the realization of new structural morphologies with acid sensitive metal oxides. Additionally, systems exist in which there is a

need to control the rate of silica hydrolysis and condensation. It needs to be very slow in order to organize/assemble nanocrystals inside a silica or other metal oxide (silsesquioxane) framework in an ordered fashion. If hydrolysis and condensation are too fast, the nanocrystal arrays become disordered. Finally, a recent review[52] highlights the importance of substrate structure on the interaction between biological materials and inorganics, suggesting applications in biology and medicine for well-ordered ceramics.

Experimental Section

Materials. The chemicals tetraethoxysilane, ethanol, and 1.0 M 9-BBN (9-borabicylco[3.3.1]nonane in THF) were purchased from Aldrich (St. Louis, MO) and used as received. Water was deionized and distilled. Ammonium molybdate was from Fisher Scientific (Fairlawn, NJ). Hydrochloric acid, anhydrous sodium sulfite, *p*-methylphenol sulfate (metol), oxalic acid dihydrate, and sulfuric acid were purchased from Acros Organics (Oxford, PA) and used as received.

Polymer Synthesis. A block copolymer of poly(butadiene-*b*-2-vinylpyridine) was synthesized using high-vacuum techniques described elsewhere.[53] The poly(hydroxylated butadiene-*b*-2-vi-nylpyridiene) copolymer was checked at each stage of synthesis by GPC. The GPC consisted of a Waters Corp. (Milford, MA) model 717 autoinjector, a Waters Corp. model 410 differential refractometer, a Waters Corp model 515 pump using THF as the mobile phase and pumping at 1 mL/min, and three 60 cm Phenomenex (Torrance, CA) Phenogel linear mixed bed GPC/SEC columns maintained at 30 °C in a column oven. A 20/10 K poly-(butadiene-*b*-2-vinylpyridine) polymer was synthesized as follows: 250 mL of a 10% benzene in cylcohexane solution degassed and dried over *sec*-butyllithium with 1,1-diphenylethylene (DPE) was distilled into a high-vacuum reaction apparatus. The initiator was 1.0 mmol of *sec*-butyllithium added by way of a break-seal. Next, 5.0 mmol of 1,2-dipiperidinoethane was added in order to give a polymer with high 1,2 microstructure. This was followed by adding 5.0 mmol of LiCl in a 0.24 M solution in THF and then adding 10 g of butadiene, which had been previously dried over *sec*-butyllithium with DPE as an indicator. After reacting for 6 h at room temperature, 5 g of 2-vinylpyridine was added. The mixture was reacted for 30 min followed by quenching with degassed methanol. The reactor was opened, and the polymer was filtered, precipitated with excess methanol, and dried in a vacuum oven. A ^{1}H NMR spectrum was then obtained for the block copolymer using a Varian Inc. (Palo Alto, CA) Mercury 300 instrument operating at 300.059 MHz.

Hydroboration. Hydroboration of double bonds is known to be very tolerant of other functional groups in the same molecule. The poly(butadiene) block of the poly(butadiene-*b*-2-vinylpyridene) was hydroborated and the borate converted to a hydroxyl functionality following previous procedures.[54] In a glovebox 1.6 g of the block copolymer (60 mmol of pendent double bonds) was added to a 500 mL round-bottom single neck flask (flask A) containing a magnetic stir bar. Next, 60 mL of 0.5 M 9-BBN solution (30 mmol) was placed in a two-neck 250 mL flask containing a magnetic stir bar (flask B), also in a glovebox. The two flasks were connected by a glass tube that contained a vacuum valve in the center. Closing the valve isolated flasks A and B. This allowed flask B to be opened and charged with reagents while avoiding exposing flask A to the atmosphere. While one neck of flask B was attached to the connecting tube, the other neck was fitted with a vacuum valve for attaching to a vacuum manifold. The apparatus (flask A containing polymer attached with a glass tube to flask B containing the 9-BBN) was removed from the glovebox and evacuated on a vacuum manifold.

THF dried over sodium and containing benzophenone as an indicator was distilled into flask A to dissolve the polymer. The polymer solution was then cooled to −20 °C and the 9-BBN solution slowly added over 1 h. After the addition of the 9-BBN, the solution temperature was allowed to rise to 0 °C, and the

reagents were allowed to react for an additional hour. The reaction temperature was then allowed to rise to room temperature, and the mixture was stirred overnight. The vacuum valve on the connecting tube was then closed, and flask B was charged with 5 mL of methanol. The methanol was degassed on the vacuum line and added by way of the connecting tube to flask A in order to quench excess 9-BNN. Flask B was then charged with 4.9 mL of 6 M NaOH. The NaOH solution was degassed and added to flask A.

Finally, 9.9 mL of 30% hydrogen peroxide in water was added to flask B. After degassing, this was added slowly to flask A, which was held at −20 °C. This addition occurred over the span of ∼2 h. After complete mixing, the reaction was left at −20 °C for 1 h, followed by allowing the reaction to come to room temperature and an additional 6 h of stirring. During the entire process, oxygen was strictly excluded from the reaction. After 6 h, the reaction mixture was poured into 1 L of methanol. This was heated slowly to remove the methanol/boric acid complex that distills at 58 °C. Removing the boric acid is critical to avoid cross-linking. The polymer was then collected and dried under vacuum. The polymer was found to be insoluble in THF but dissolved in a 2:1 solution of water:isopropanol (by volume). GPC analysis of the polymer using a 3:2 water:isopropanol solution as the mobile phase showed a single peak with no indication of cross-linking. The GPC consisted of a Waters Corp. model 515 pump operating at 0.8 mL/min, a Waters Corp. model 410 differential refractometer, a Waters Corp. manual injector, and two 60 cm Linear Polysep GFC-P columns from Phenomenex. ^{1}H NMR spectra were obtained of the polymer. A Varian Mercury 300 NMR was used to obtain ^{1}H NMR of both the functionalized and unfunctionalized polymer.

Molybdic Acid Assay. The molybdic acid assay procedure was based on the UV−vis absorption of a reduced silicomolybdate complex to determine the concentration of silicon in solution.[55,56] The block copolymer was placed in a 2 mL centrifuge tube and dissolved in 0.6 mL of a 1:1 by weight water:ethanol solution. This solution was allowed to stand overnight to be sure of complete dissolution. 1 mL of TEOS was then added. The sample was mixed by vortex and then agitated for 1 h on a shaker plate. The samples were then centrifuged for 2 h to force any precipitate to the bottom of the tube. The supernatant was then poured off and replaced by pure ethanol. This mixture was then centrifuged and the supernatant poured off to remove any unreacted TEOS. A solid precipitate of the polymer and reacted TEOS remained in the tube. This material was analyzed by a modification of a molybdic acid assay as described in the following paragraphs.

The molybdic acid reagents used for assay were prepared according to a literature procedure.[57] Two solutions were prepared. The first contained 41.0 mL of 95.5% sulfuric acid diluted to 1 L to make a 1.5 N solution. The second contained 100 g of ammonium molybdate (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O) and 47 mL of concentrated ammonium hydroxide diluted with water to 1 L. To form the silicomolybdic acid, 200 mL of the acid solution was added to 500 mL of water followed by 100 mL of the molybdate solution. This solution was used within 1 week of its preparation.

Because of the instability of the silicomolybdic acid, the solutions were reduced using metol (*p*-(methylamino)phenol sulfate) prior to absorbance measurements. Three solutions were prepared for this step. First, 6 g of anhydrous sodium sulfite was added to 500 mL of distilled water, to which 10 g of metol was then added. After all the reagents appeared to dissolve, the solution was filtered and stored. The second solution contained 50 g of oxalic acid dihydrate in 500 mL of water. This was a saturated solution and was decanted from the undissolved oxalic acid prior to use. The third solution was 250 mL of concentrated sulfuric acid diluted to 500 mL. For the reducing solution, 100 mL of the metol solution was mixed with 60 mL of the oxalic acid solution. This was followed by the addition of 60 mL of the sulfuric acid solution, and the entire mixture was diluted to 300 mL with distilled water. This solution was used immediately.

The centrifuge tube containing the polymer/TEOS precipitate was rinsed several times with a 1 M NaOH solution to remove all silicate. These rinses were combined in a 50 mL volumetric flask,

and to this vessel 10 mL of the molybdic acid solution described above was added. The mixture was allowed to react for ∼10 min, and the solution became yellow. The reducing solution made from the metol and oxalic acid was added to the yellow solution, changing the color to blue. An aliquot was removed from the now blue solution, placed in a 1 cm quartz cell, and allowed to stand for 2−3 h. The absorption at 810 nm was then measured using a Agilent Technologies model 8453 UV−vis spectrometer (Palo Alto, CA). (The blue solution was stable for at least 6 h.)

The molybdic acid assay was calibrated by the use of Na$_2$SiF$_6$. The solution used for calibration consisted of 0.960 g of Na$_2$SiF$_6$ diluted to 1000 mL. This yielded a solution containing 5 μg of silica/mL of solution. The silica was very stable in this form and could be stored indefinitely.

Thermal Gravitational Analysis. TGA was used to determine the amount of solid poly(silicate) formed relative to the amount of polymer employed. Measurements were taken on a sample of the solid isolated by centrifugation and washing to remove unreacted TEOS. Simultaneous TGA and differential scanning calorimetry (DSC) were performed using a Rheometrics, Inc. (Piscataway, NJ) model STA 1500C simultaneous thermal analyzer. Samples were heated from ambient to just below 100 °C (10 °C/min) and held there for 1 h to dry, then heated slowly (1 °C/min) to 600 °C, and held at that temperature for several hours to remove both the organic and other volatile components.

Scanning Electron Microscopy. SEM on metal-coated silica particles was performed using a Philips (Eindhoven, The Netherlands) model XL-30 FEG-SEM under an accelerating voltage of 10 keV. Dried particles were adhered to aluminum stub mounts using carbon tape and then coated with ∼1.0 nm thick titanium using a South Bay Technology, Inc. (San Clemente, CA) model VCR IBS/TM250 ion beam sputterer.

Solid-State Nuclear Magnetic Resonance. The silicon-29 (^{29}Si) solid-state magic angle spinning (MAS) nuclear magnetic resonance spectra spectrum was acquired using an Inova 400 MHz wide-bore spectrometer (Varian; Palo Alto, CA) operating at 79.42 MHz. A quantitative ^{29}Si NMR MAS spectrum was obtained using a single 45° rf pulse (3.5 μs) separated by 300 s recycle delays. The spectral width was 50 kHz, and a total of 2000 transients were acquired to obtain a reasonable signal-to-noise ratio. The spinning rate was 5 kHz at room temperature using a 7.5 mm zirconia rotor with a Chemagnetics NMR probe (Otsuka Electronics USA, Fort Collins, CO). Chemical shifts are given in ppm from tetramethylsilane (TMS).

Fourier Transform Infrared Spectroscopy. FTIR spectroscopic analysis was used to measure the increase in ethanol content in ethanol/water/TEOS solutions, based on the production of ethanol as the byproduct of TEOS hydrolysis. A time series of ATR infrared measurements were made on solutions in contact with a ZnSe crystal used as the ATR guide.

All solutions were prepared using a solvent of ethanol (reagent grade, Sigma-Aldrich, St. Louis, MO) and water (1:1 by volume). In order to remain consistent, all studies of the functionalized polymer, including molybdic acid assays and control reactions, were carried out using the 50:50 ethanol:water solution as solvent. TEOS is not miscible with water and so requires the addition of the cosolvent ethanol to ensure a homogeneous reaction. The catalyst− polymer, acid, base, or surfactant/acid−was mixed into solution with the ethanol/water solvent. Hydrochloric acid and sodium hydroxide (Sigma-Aldrich, St. Louis, MO) were used as the respective acid and base source and were added dropwise to a pH of 2 in the acid-catalyzed solution and a pH of 10 in the basic solution. The surfactant/acid system was the same described in Yao et al. and consisted of the surfactant cetyltrimethylammonium chloride (CTAC, Sigma-Aldrich, St. Louis, MO) and hydrochloric acid, the latter added in sufficient quantity to lower the solution pH to 2.[51] After preparing the catalyst solution, TEOS (reagent grade, Fluka, Sigma-Aldrich, St. Louis, MO) was added in amounts slightly below the molar ratio of 4:1 water:TEOS, based on the water content of the solution, to ensure complete hydrolysis of the TEOS. Immediately following TEOS addition, the solution was

simply poured into a horizontal trough whose bottom surface was a ZnSe crystal (bevel angle of 45°), covered, and sealed against atmosphere and evaporation during measurement. The rate of TEOS hydrolysis is relatively slow, affording ample time to transfer solution from mixing vessel to the sampling chamber.

Continuous ATR spectra were recorded using a Smart Ark horizontal ATR with trough insert on a Nexus 670 FTIR spectrophotometer (Thermo Nicolet, Madison, WI). Time stamps and spectra were recorded using a closed loop macro program. Full mid-infrared spectra ($400-4000$ cm^{-1}) were recorded at 90 min intervals for the first 12 h and at 180 min intervals thereafter. To increase the signal/noise ratio, each spectrum was the average of 512 scans. The data acquisition and subsequent analysis were done using the OMNIC spectral analysis software (Thermo Nicolet, Madison, WI).

References and Notes

(1) Aelion, R.; Loebel, A.; Eirich, F. *J. Am. Chem. Soc.* **1950**, *72*, 5705−5712.

(2) Colby, M. W.; Osaka, A.; Mackenzie, J. D. *J. Non-Cryst. Solids* **1986**, *82*, 37−41.

(3) See, for example: Klein, L. S. *Annu. Rev. Mater. Sci.* **1985**, *15*, 227−248.

(4) Brinker, C. J.; Scherer, G. W. *Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing*; Academic Press: New York, 1990; Chapter 3.

(5) Stöber, W.; Fink, A.; Bohn, E. *J. Colloid Interface Sci.* **1968**, *26*, 62−69.

(6) See, for example: Belcher, A. M.; Wu, X. H.; Christensen, R. J.; Hansma, P. K.; Stucky, G. D.; Morse, D. E. *Nature (London)* **1996**, *381*, 56−58.

(7) Mann, K.; Weiss, I. M.; André, S.; Gabius, H.; Fritz, M. *Eur. J. Biochem.* **2000**, *267*, 5257−5264.

(8) Weiss, I. M.; Göhring, W.; Fritz, M.; Mann, K. *Biochem. Biophys. Res. Commun.* **2001**, *285*, 244−249.

(9) Dabbs, D. M.; Aksay, I. A. *Annu. Rev. Phys. Chem.* **2000**, *51*, 601−622.

(10) McGrath, K. M.; Dabbs, D. M.; Yao, N.; Aksay, I. A.; Gruner, S. *Science* **1998**, *277*, 552−556; *279*, 1289.

(11) McGrath, K. M.; Dabbs, D. M.; Yao, N.; Edler, K. J.; Aksay, I. A.; Gruner, S. M. *Langmuir* **2000**, *16*, 398−406.

(12) Morse, D. E. *Trends Biotechnol.* **1999**, *17*, 230−232.

(13) Morse, D. E. In *The Chemistry of Organic Silicon Compounds*; Rappoport, Z., Apeloig, Y., Eds.; John Wiley & Sons: New York, 2001; Vol. 3, p 805.

(14) See, for example: Vartuli, J. C.; Schmitt, K. D.; Kresge, C. T.; Roth, W. J.; Leonowicz, M. E; McCullen, S. D.; Beck, J. S.; Schlenker, J. L.; Olson, D. H.; Sheppard, E. W. *Chem. Mater.* **1994**, *6*, 2317−2326.

(15) Huo, Q.; Margolese, D. I.; Ciesla, U.; Feng, P.; Gier, T. E.; Sieger, P.; Leon, R.; Petroff, P. M.; Schuth, F.; Stucky, G. D. *Nature (London)* **1994**, *368*, 317−321.

(16) Tanev, P. T.; Pinnavaia, T. J. *Science* **1995**, *267*, 865−867.

(17) Shimizu, K.; Cha, J.; Stucky, G. D.; Morse, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6234−6238.

(18) Cha, J. N.; Shimizu, K.; Zhou, Y.; Christiansen, S. C.; Chmelka, B. F.; Stucky, G. D.; Morse, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 361−365.

(19) Zhou, Y.; Shimizu, K.; Cha, J. N.; Stucky, G. D.; Morse, D. E. *Angew. Chem., Int. Ed.* **1999**, *38*, 780−782.

(20) Sumerel, J. L.; Yang, W. J.; Kisailus, D.; Weaver, J. C.; Choi, J. H.; Morse, D. E. *Chem. Mater.* **2003**, *15*, 4804−4809.

(21) Karp, G. *Cell Biology*; McGraw-Hill: New York, 1984; p 286.

(22) Cha, J. N.; Stucky, G. D.; Morse, D. E.; Deming, T. J. *Nature (London)* **2000**, *403*, 289−292.

(23) Mizutani, T.; Nagase, H.; Ogoshi, H. *Chem. Lett.* **1998**, *2*, 133−134.

(24) Mizutani, T.; Nagase, H.; Fujiwara, N.; Ogoshi, H. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2017−2022.

(25) Coradin, T.; Livage, J. *Colloids Surf., B* **2001**, *21*, 329−336.

(26) Coradin, T.; Durupthy, O.; Livage, J. *Langmuir* **2002**, *18*, 2331−2336.

(27) Patwardhan, S. V.; Clarson, S. J. *Polym. Bull. (Berlin)* **2002**, *48*, 367−371.

(28) Patwardhan, S. V.; Mukherjee, N.; Steinitz-Kannan, M.; Clarson, S. J. *Chem. Commun.* **2003**, *10*, 1122−1123.

(29) Patwardhan, S. V.; Clarson, S. J. *Silicon Chem.* **2002**, *1*, 207−214.

(30) Roth, K. M.; Zhou, Y.; Yang, W. J.; Morse, D. E. *J. Am. Chem. Soc.* **2005**, *127*, 325−330.

(31) Kisailus, D.; Najarian, M.; Weaver, J. C.; Morse, D. E. *Adv. Mater.* **2005**, *17*, 1234−1239.

(32) van Bommel, K. J. C.; Shinkai, S. *Langmuir* **2002**, *18*, 4544−4548.

(33) Bates, F. S.; Fredrickson, G. H. *Annu. Rev. Phys. Chem.* **1990**, *41*, 525−557.

(34) Zhao, D. Y.; Feng, J. L.; Huo, Q. S.; Melosh, N.; Fredickson, G. H.; Chmelka, B. F.; Stucky, G. D. *Science* **1998**, *279*, 548−52.

(35) Zhao, D. Y.; Huo, Q. S.; Feng, J. L.; Chmelka, B. F.; Stucky, G. D. *J. Am. Chem. Soc.* **1998**, *120*, 6024−36.

(36) Melosh, N. A.; Lipic, P.; Bates, F. S.; Wudl, F.; Stucky, G. D.; Fredrickson, G. H.; Chmelka, B. F. *Macromolecules* **1999**, *32*, 4332−4342.

(37) Innocenzi, P.; Falcaro, P.; Grosso, D.; Babonneau, F. *J. Phys. Chem. B* **2003**, *107*, 4711−4717.

(38) Chow, D. T.-W.; Robinson, R. J. *Anal. Chem.* **1953**, *25*, 646−648.

(39) Alexander, G. B. *J. Am. Chem. Soc.* **1953**, *75*, 5655−5657.

(40) Alexander, G. B. *J. Am. Chem. Soc.* **1954**, *76*, 2094−2096.

(41) Brinker, C. J.; Drotning, W. D.; Scherer, G. W. In *Better Ceramics through Chemistry*; Mater. Res. Soc. Symp. Proc. 32; Brinker, C. J., Ulrich, D. R., Clark, D. E., Eds.; Elsevier Science Publishing: St. Louis, MO, 1984; pp 25−32.

(42) Treadwell, D. R.; Dabbs, D. M.; Aksay, I. A. *Chem. Mater.* **1996**, *8*, 2056−2060.

(43) Nakajima, T.; Matsuoka, M.; Mishima, S.; Matsuzaki, I. *Nippon Kagaku Kaishi* **1994**, *12*, 1134−1136.

(44) Koch, C. C. *Nanostructured Materials*; Noyes Publications: Norwich, NY, 2002; Chapter 1.

(45) Thommes, M.; Smarsly, B. Groenewolt, M.; Ravikovitch, P. I.; Neimark, A. V. *Langmuir* **2006**, *22*, 756−764.

(46) Artaki, I.; Bradley, M.; Zerda, T. W.; Jonas, J. *J. Phys. Chem.* **1985**, *89*, 4399−4404.

(47) Liu, C. C.; Maciel, G. E. *J. Am. Chem. Soc.* **1996**, *118*, 5103−5119.

(48) Grimmer, A. R.; Rosenberger, H.; Bürger, H.; Vogel, W. *J. Non-Cryst. Solids* **1988**, *99*, 371−378.

(49) Gallignani, M.; Ayala, C.; Brunetto, M. D.; Burguera, J. L.; Burguera, M. *Talanta* **2005**, *68*, 470−479.

(50) Garrigues, J. M.; Perez-Ponce, A.; Garrigues, S.; de la Guardia, M. *Vib. Spectrosc.* **1997**, *15*, 219−228.

(51) Yao, N.; Ku, A. Y.; Nakagawa, N.; Lee, T.; Saville, D. A.; Aksay, I. A. *Chem. Mater.* **2000**, *12*, 1536−548.

(52) Stevens, M. M.; George, J. H. *Science* **2005**, *310*, 1135−1138.

(53) Morton, M.; Fetters, L. J. *Rubber Chem. Technol.* **1975**, *48*, 359−365.

(54) Wang, J.; Mao, G.; Ober, C. K.; Kramer, E. J. *Macromolecules* **1997**, *30*, 1906−1914.

(55) Strickland, J. D. H.; Parsons, T. R. In *A Practical Handbook of Seawater Analysis*, 2nd ed.; Fisheries Research Board of Canada: Ottawa, 1972; Chapter II.5.

(56) Brzezinski, M. A.; Nelson, D. M. *Mar. Chem.* **1986**, *19*, 139−151.

(57) Iler, R. K. *The Chemistry of Silica*; John Wiley and Sons: New York, 1979; p 96.