

Bioinspired Insights into Silicic Acid Stabilization Mechanisms: The Dominant Role of Polyethylene Glycol-Induced Hydrogen Bonding

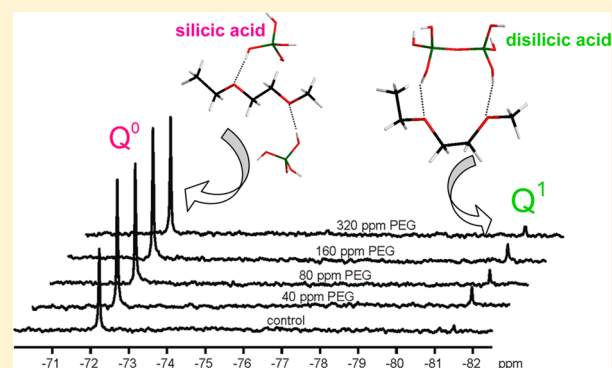
Melina Preari,[†] Katrin Spinde,[‡] Joëlle Lazic,[‡] Eike Brunner,[‡] and Konstantinos D. Demadis^{*,†}

[†]Crystal Engineering, Growth and Design Laboratory, Department of Chemistry, University of Crete, Voutes Campus, Heraklion, Crete, GR-71003, Greece

[‡]Fachrichtung Chemie und Lebensmittelchemie, Bioanalytische Chemie, Technische Universität Dresden, 01062 Dresden, Germany

S Supporting Information

ABSTRACT: Mono- and disilicic acids were stabilized by uncharged polyethylene glycols (PEGs) in silica-supersaturated solutions (the starting solution contained 500 ppm/8.3 mM sodium orthosilicate, $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$, expressed as SiO_2) at pH = 7, most likely by hydrogen bonding between the silanol groups and $-\text{CH}_2-\text{CH}_2-\text{O}-$ ether moieties. The stabilization was monitored by measuring molybdate-reactive silica and also by a combination of liquid- and solid-state ^{29}Si NMR spectroscopy. It depends on PEG concentration (20–100 ppm) and molecular weight (1550–20 000 Da). Two narrow ^{29}Si NMR signals characteristic for monosilicic acid (Q^0) and disilicic acid (Q^1) can be observed in ^{29}Si NMR spectra of solutions containing PEG 10000 with intensities distinctly higher than the control, that is, in the absence of PEG. Silica-containing precipitates are observed in the presence of PEG, in contrast to the gel formed in the absence of PEG. These precipitates exhibit similar degrees of silica polycondensation as found in the gel as can be seen from the ^{29}Si MAS NMR spectra. However, the ^2D HETCOR spectra show different ^1H NMR signal shifts: The signal due to H-bonded $\text{SiOH}/\text{H}_2\text{O}$, which is found at 6 ppm in the control, is shifted to ~ 7 ppm in the PEG-containing precipitate. This indicates the formation of slightly stronger H-bonds than in the control sample, most likely between PEG and the silica species. The presence of PEG in these precipitates is unequivocally proven by ^{13}C CP MAS NMR spectroscopy. The ^{13}C signal of PEG significantly shifts and is much narrower in the precipitates as compared to the pristine PEG, indicating that PEG is embedded into the silica or at least bound to its surface (or both), and not phase separated. FT-IR spectra corroborate the above arguments. The H-bonding between silanol and ethereal O perturbs the band positions attributed to vibrations involving the O atom. This work may invoke an alternative way to envision silica species stabilization (prior to biosilica formation) in diatoms by investigating possible scenarios of uncharged biomacromolecules playing a role in biosilica synthesis.



INTRODUCTION

Silicon is abundant in the Earth's crust. Several organisms use it mostly in the form of silica and are thus part of the global Si cycle.^{1–3} Si metabolism of such organisms is a remarkable source of inspiration for bioinorganic and biomimetic chemistry. One of the most spectacular examples of such silica biomineralizing organisms are diatoms that are unicellular algae living in fresh and marine water habitats.^{4–7} Diatoms preferentially take up “Si” as monosilicic acid ($\text{Si}(\text{OH})_4$)^{8,9} via special “Si” transport (SIT) proteins.^{10–12} Active transport is necessary because the concentration of silicic acid in natural waters is low.^{13–15} The SIT proteins determine “Si” uptake at low $\text{Si}(\text{OH})_4$ concentrations, whereas the uptake is diffusion-controlled at high $\text{Si}(\text{OH})_4$ levels.^{16,17} However, the intracellular “Si” processing, transport, and transfer into the silica deposition vesicle (SDV) are rather poorly understood.^{17–21} Numerous publications report the existence of intracellular “Si” storage pools in diatoms. “Si” storage pools in diatom cells, if present, provide “Si” for the production of new valves.^{22,23} The

reported concentrations up to ca. 300 mM intracellular “Si” within the storage pool^{24–28} strongly exceed the solubility of monosilicic acid (~ 2 mM/150 ppm, pH < 9).^{29–31} “Si” species are assumed to be stabilized via association with “organic material” such as special proteins, thus forming intracellular soluble $\text{Si}(\text{OH})_4$ pools.³² Special “Si” transport vesicles (STVs) were proposed.³³ However, no evidence for the presence of “Si” inside the diatom STVs exists, in contrast to sponges.³⁴ Various biomacromolecules such as special proteins,^{35–39} long-chain polyamines,^{40,41} and polysaccharides^{42–44} have been found as integral parts of the cell wall. These molecules are assumed to play a role in silica biogenesis.

^{29}Si MAS NMR spectroscopy has been widely used to characterize siliceous materials⁴⁵ revealing the different Q^n group species (Q^n : $\text{Si}(\text{OSi})_n(\text{OH})_{4-n}$, $n = 0–4$). It could be applied to study extracted diatom biosilica^{46,47} as well as intact

Received: November 20, 2013

Published: February 24, 2014

diatom cells after isotope enrichment.^{48,49} Liquid-state ²⁹Si HR NMR spectroscopy has previously been used to study silicic acid polycondensation in vitro^{50,51} and in diatom cells without chemical pretreatment.⁵² The idea of Si(OH)₄ stabilization by “organic material” has meanwhile led to various in vitro investigations to model the influence of biomolecules found in diatoms on Si(OH)₄ chemistry. ²⁹Si NMR revealed^{53–55} that carbohydrate-like molecules can interact in vitro with silicate, forming stable 5- and 6-coordinated complexes, using Na-silicate as the Si source. Representative conditions, as stated by Kinrade et al.,^{53–55} include pH = 7, 1.2–1.4 mol kg⁻¹ SiO₂, 2.9 mol kg⁻¹ NaOH, at 270 K. Polysaccharides have indeed been found attached to diatom biosilica.^{42–44}

In diatoms, long-chain polyamines are found.^{40,41} They may also be covalently attached to lysine residues in proteins, coined silaffins.^{35,36} Sumper proposed the presence of so-called polyamine-stabilized silica sols as silica precursors,⁵⁶ which are stable over 24 h. Rapid silica precipitation only occurs in the presence of appropriate counterions such as phosphate^{57,58} or the highly phosphorylated silacidins.^{37,38}

With respect to the type of stabilized “Si” species, two different possibilities are envisioned: (i) mono- and disilicic acid (Q⁰ + Q¹); and (ii) higher silicic acid oligomers/silica nanoparticles (Q², Q³, Q⁴). The idea of polyamine-stabilized silica sols has spurred several in vitro studies on the interaction of silicic acid with polymeric chains. Polymers such as poly(vinylamine),⁵⁹ NH₂-terminated polyaminoamide dendrimers,^{60–63} phosphonate-grafted chitosan zwitterionic derivatives,^{31,64} and other amine- (or ammonium)-based synthetic polymers⁶⁵ were investigated by the silicomolybdate test detecting the total amount of soluble species (Q⁰ + Q¹). So far, in vitro Si(OH)₄ stabilization with the “intervention” of purposely added polymers has led to silicic acid levels up to ~8 mM. It is, therefore, important to gain further insight into the interaction between polymers and silicic acid species in solution at a molecular level, to mimic the extraordinary ability of the diatom to stabilize extreme levels of uncondensed Si(OH)₄. Our research thus far revealed that polymers possessing protonated primary, secondary, or tertiary amine groups, organo-ammonium groups,^{31,60–65} amide moieties,⁶⁶ and, surprisingly, polymer-grafted phosphonium groups,⁶⁷ can stabilize Si(OH)₄.

Apart from electrostatic interactions, H-bonding is another important interaction capable of influencing silica formation.^{68–70} To eliminate the influence of electrostatic interactions, the effect of H-bonding should be investigated using uncharged molecules. An ideal candidate is polyethylene glycol (PEG). Si(OH)₄ is also uncharged under conditions relevant for our study (circumneutral pH). PEG is well-known to interact with water and other molecules via H-bonding,^{71,72} resulting in an increasing viscosity of aqueous PEG solutions as compared to pure water. Long-chain PEGs exhibit a stronger influence upon the viscosity than do short-chain PEGs.^{73–75} It is reasonable to assume that H-bonding is the most relevant molecular interaction pathway between PEG and the OH groups of the mainly undissociated Si(OH)₄, whereas hydrophobic interactions, another potentially important silica-organic interaction, can be safely neglected. Even if the ethylene (–CH₂CH₂–) moieties in PEG could induce hydrophobic interactions, these can only be imagined with other ethylene (–CH₂CH₂–) moieties of PEG chains, or maybe colloidal silica. Hydrophobic interactions may, therefore, be important at later stages of the silica formation as discussed

by Vrieling and co-workers.⁷⁰ The aim of this study is the systematic analysis of the influence of six “simple” PEG polymers of different molecular weight (MW) between 1550–20 000 Da upon the Si(OH)₄ polycondensation process. Two approaches were followed to study the stabilization effect. “Short-term” (for 8 h) and “long-term” (for at least 1 and up to 3 days) experiments were implemented to study the condensation reaction.

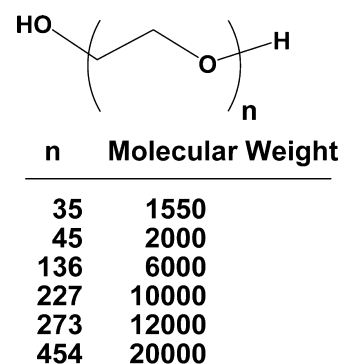
EXPERIMENTAL SECTION

Definitions and Si Species Nomenclature. Icopini et al.⁷⁶ state that “Iler”⁷⁷ and others have argued that the silicomolybdate method used within the present work (see below) detects different low-weight silica polymers, including monomeric (H₄SiO₄), dimeric (H₆Si₂O₇), and possibly trimeric (H₈Si₃O₁₀) silica. Comparisons of [SiO₂]_{TD} (TD = total dissolved) and [SiO₂]_{n≤3} indicated that the stock solution at pH 11 consisted only of molybdate-reactive silica.”

We, henceforth, agree with the definition of Icopini et al. and adopt the terminology “molybdate-reactive” in this Article. We define “molybdate-reactive” as the “Si” species that are responsive to the silicomolybdate test, described in detail below. In accordance with the above-mentioned definition of Icopini et al., “molybdate-reactive” includes all three species, monomeric (monosilicic acid, H₄SiO₄), dimeric (disilicic acid, H₆Si₂O₇), and possibly trimeric (H₈Si₃O₁₀) silica. However, because we did not detect trimeric silica in any of our experiments (see below), the term “molybdate-reactive” silica refers to a mixture of monosilicic acid (primarily) and disilicic acid, with the first being dominant under our experimental conditions.

Reagents, Chemicals, and Materials. Sodium silicate Na₂SiO₃·5H₂O was purchased from EM Science (Merck). Solid samples of PEG polymers were commercial samples. PEG 1550, PEG 6000, and PEG 20000 from SERVA Electrophoresis GmbH (Germany), and PEG 2000, PEG 10000, and PEG 12000 from Alfa Aesar (U.S.). They were used as received. Some information about them is given in Scheme 1.

Scheme 1. Basic Structure, MWs, and Number of O Atoms per PEG Polymer Used in This Work



Ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O) and oxalic acid (H₂C₂O₄·2H₂O) were obtained from EM Science (Merck). Sodium hydroxide (NaOH) was purchased from Merck, and hydrochloric acid 37% was from Riedel de Haen. In-house deionized water from an ion-exchange resin was used for all experiments. This water was tested for molybdate-reactive silica and was found to contain negligible amounts. Acrodisc filters (0.45 μm and occasionally 0.20 μm) from Pall-Gelman Corp. were used.

Preparation of Supersaturated Sodium Silicate Solutions. A solution containing silicate (500 ppm as SiO₂) was prepared by dissolving 4.4 g of Na₂SiO₃·5H₂O in 2.5 L of nanopure water (a nonglass container must be used), followed by overnight rigorous stirring. This solution contains exclusively molybdate-reactive silica in agreement with the literature.^{77,78} Stock solutions of the additives in water were 1% w/v (10 000 ppm). The following solutions were

prepared for the silicate spectrophotometric detection test: (a) 10 g of ammonium molybdate was dissolved in 100 mL of water, and its pH was adjusted between 7 and 8 with NaOH to avoid precipitation of ammonium molybdate. (b) HCl 1 + 1 was prepared by mixing one volume 37% HCl with equal volume water. (c) 8.75 g of oxalic acid was dissolved in 100 mL of water. All solutions were kept in polyethylene containers (glass containers must be avoided to minimize SiO₂ dissolution and silicate leaching into the test solutions).

Silicic Acid Polycondensation Protocol. Silicic Acid Polycondensation in the Absence of PEGs ("Control" Protocol). 100 mL from the 500 ppm sodium silicate stock solution (see above) was placed in a polyethylene beaker, which was charged with a Teflon-covered magnetic stirring bar. The pH of this solution was initially ~11.8 and is subsequently adjusted to 7.00 ± 0.1 by addition of HCl and NaOH (the change in the resulting volume was about 3% and was taken into account for subsequent calculations). The beaker was then covered with plastic membrane and set aside without stirring. The solutions were checked for molybdate-reactive silica by the silicomolybdate method every 1 hour for the first 8 hours or after 24, 48, 72 h time intervals after the onset of the pH adjustment to 7.0. There must be strict time control in measuring molybdate-reactive silica, to avoid conversion of higher oligomers/colloidal silica to silicic and disilicic acids. Specifically, after the ammonium molybdate and HCl solutions were added to a working sample, a period of 10 min has to pass until the solution of oxalic acid is added to the same sample. Another 2 min period then has to follow until the final measurement. All samples (control and in the presence of PEGs) were treated in precisely the same way. Separate experiments were performed in which the working solutions were stirred, but no difference in molybdate-reactive silica levels was found, as compared to the quiescent solutions.

Silicic Acid Polycondensation in the Presence of PEGs. 100 mL portions of the 500 ppm sodium silicate stock solution (see above) were placed in polyethylene containers and charged with Teflon-covered magnetic stir bars. In each container, different volumes of PEG (from the prepared 10 000 ppm stock solutions) were added to achieve desirable PEG concentration. These ranged from 20–40–60–80–100 ppm, and the added volumes were 200–400–600–800–1000 μL. After that, the same procedure as the "control" protocol was followed.

Determination of Molybdate Reactive Silica. Frequently, there is a misconception about the use of the terms "silica", "silicate", and "silicic acid". In this Article, the term "silica" indicates the product of silicic acid polycondensation. The term "silicate" indicates all of the forms of the species Si(OH)₄ at various deprotonated states (Si(OH)_{4-x}^{-x}, x = 0, 1, 2, 3, 4; 4 indicates that silicic acid is fully deprotonated), which exist at pH regions >9.⁷⁹ Note that mono- and disilicic acid rapidly interconvert, and they both contribute to molybdate-reactive silica. Molybdate-reactive silica was quantified using the well-established silicomolybdate spectrophotometric method. For our studies, we used a modification of (several) published procedures (for which a precision of ±5% is reported),^{80–85} as follows: A quantity of 2 mL aliquot from the working solution is filtered through a 0.45 μm syringe filter, and then diluted to 25 mL in a special cylindrical cell (the Hach Co.) of 1 cm path length. Next, 1 mL of ammonium molybdate stock solution and 0.5 mL of HCl (1:1 dilution of the concentrated solution) are added to the sample cell, the solution is mixed well by shaking, and finally left undisturbed for 10 min. After that, 1 mL of oxalic acid stock solution is added and thoroughly mixed again. The solution is set aside for 2 min. After the second time period, the photometer is set to "zero absorbance" using a sample of water plus all chemicals used for the silicomolybdate test except for silicic acid ("blank"). Finally, the sample absorbance is measured (at 420 nm) and is expressed as "ppm SiO₂". The detectable concentration range for this specific protocol is 6–75 ppm. To calculate the concentration in the original solution, an appropriate dilution factor is applied.

The silicomolybdate method is based on the principle that ammonium molybdate reacts only with "reactive" silica (see definitions above) and any phosphate present at low pH (about 1.2) and yields heteropoly acids, yellow in color. Oxalic acid is added to destroy the

molybdophosphoric acid (in case phosphate is present in the water), leaving the silicomolybdate complex intact, and thus eliminating any color interference from phosphates. It should be emphasized that molybdate reacts only with the monomeric, dimeric, and possibly trimeric forms as stated above, but is totally unreactive to colloidal silica particles, if the measurements are performed with strict time control (see above). This was verified experimentally in our laboratory. Within the strict time control of the above-described measurements, only mono- and disilicic acids are reactive.

Reagents, Materials, and Procedures for NMR Studies. Materials for NMR Studies. All reagents used in this work were purchased from commercial sources. The ²⁹Si-enriched samples were prepared using 96.74% ²⁹Si-enriched sodium metasilicate (Na₂²⁹SiO₃). To obtain the latter, ²⁹SiO₂ (CortecNet, France) was melted with sodium carbonate (Fluka), thus forming Na₂²⁹SiO₃ in a solid-state reaction. For the samples studied by NMR, the total amount of molybdate-reactive silica was determined by the silicomolybdate test (molybdenum blue method, in this case) following our protocol previously described in detail.⁶⁶

Sample Preparation for ²⁹Si HR NMR and MAS NMR. Following the protocol described previously,⁶⁶ we have prepared stock solutions of 6030 ppm ²⁹SiO₂ and 600 ppm ²⁹SiO₂ for solid-state MAS NMR and HR NMR, respectively. For the control samples, nanopure water was added to the stock solution, thus giving the desired starting concentration of ²⁹SiO₂ (500 ppm for HR NMR, 4200 ppm for solid-state MAS NMR). The pH was adjusted with a 2.4 M HCl stock solution to 7.0 ± 0.1. The additive-containing samples were also prepared from the aforementioned stock solutions. Additive-containing stock solutions + nanopure water were admixed to give the desired concentrations of additive and SiO₂. Titration to the desired pH was again performed with 2.4 M HCl solution. The kinetics of silicic acid polycondensation was studied by liquid-state ²⁹Si NMR. After a reaction time of 24 h, the obtained gel/precipitates were characterized by solid-state ²⁹Si MAS NMR.

²⁹Si and ¹³C NMR Spectroscopy. ²⁹Si NMR experiments were performed on a Bruker Avance 300 spectrometer operating at a resonance frequency of 59.63 MHz. For liquid-state ²⁹Si NMR measurements, a commercial 10 mm HR probe was used (56° flip angle, 60 s repetition time, typical T₁ values for samples in solution were 8–13 s). Waltz16 ¹H-decoupling was applied during signal acquisition. The chemical shift was referenced relative to TMS, and the quantitative measurements were calibrated using a 4200 ppm sodium metasilicate solution at pH > 11 as an external standard. ²⁹Si MAS NMR measurements were carried out on commercial double resonance 4 and 2.5 mm MAS NMR probes. SPINAL64 ¹H-decoupling was applied during signal acquisition. ¹³C CP MAS NMR spectra were acquired at 201.19 MHz using ramped ¹H–¹³C cross-polarization (CP) on a Bruker Avance 800 spectrometer.

RESULTS AND DISCUSSION

First, we have investigated the influence of the PEG polymer chain length and concentration. In the absence of PEG polymers, 188 ppm of molybdate-reactive silica (38% of the initial amount) is quantified after 8 h (Figure 1). PEG 1550 (i.e., PEG with a MW of 1550) at 80 ppm induces stabilization, resulting in 243 ppm molybdate-reactive silica (49% of the initial amount).

Further MW increase (PEG 2000) causes an increasing stabilization resulting in 287 ppm molybdate-reactive silica, that is, an additional 44 ppm as compared to PEG 1550. There seems to be an upward trend in stabilization with MW increase. However, the stabilization activity is indistinguishable above 10 000 Da (for all 8-h results, see Supporting Information Figure S-1). About 360–370 ppm molybdate-reactive silica is then observed for all PEGs with MW ≥ 10 000.

Figure 2 displays silicic acid levels in the presence of various PEGs after 24 h. In these "long-term" experiments, the dependence of stabilization on MW is clearly evident. For

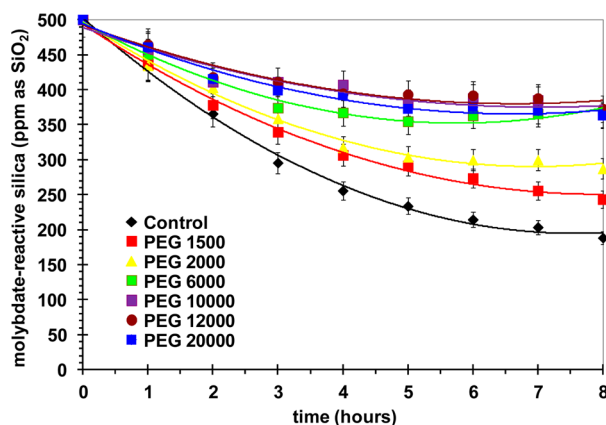


Figure 1. Stabilization of molybdate-reactive silica by PEG polymers during the first 8 h of the condensation reaction. All polymer concentrations are 80 ppm (pH 7.0).

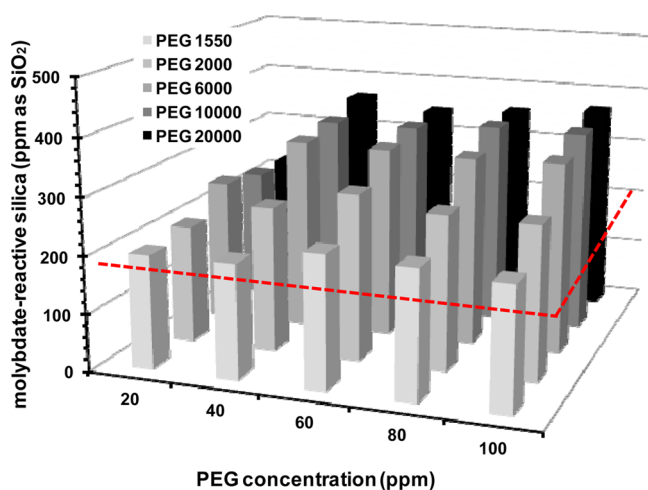


Figure 2. Stabilization of molybdate-reactive silica with PEG polymers (after 24 h, pH 7.0). The red dotted line represents the level of molybdate-reactive silica in the absence of PEGs and is added to guide the eye. The bars have been drawn at a gradually darker scale to denote increase in PEG MW.

example, PEG 20000 is capable of stabilizing 367 ppm molybdate-reactive silica (73% of the initial amount) after 24 h (for the entire set of 3-day results, see Supporting Information Figure S-2). It is notable that the amount of stabilized molybdate-reactive silica levels off at a certain PEG concentration. This indicates that the effect cannot simply be due to increasing solution viscosity. Increasing PEG concentration will lead to a steadily increasing viscosity. This, in turn, slows diffusion of $\text{Si}(\text{OH})_4$, leading to a lower probability for collisions between $\text{Si}(\text{OH})_4$ molecules. If viscosity-limited diffusion would be the only effect, an increasing PEG concentration should result in steadily decreasing polycondensation rates, in contrast to the observed behavior.

Significant stabilization of molybdate-reactive silica could also be monitored with liquid-state ^{29}Si NMR at pH 7, Figures 3 and 4 in combination with the molybdenum blue test (see Table 1 and Supporting Information Figure S-3).

Figure 3 displays the ^{29}Si NMR spectra of two samples containing an initial silicic acid concentration of 500 ppm without (control) and with 40 ppm PEG 10000 at pH 7 measured after 2 days reaction time. Two narrow ^{29}Si NMR

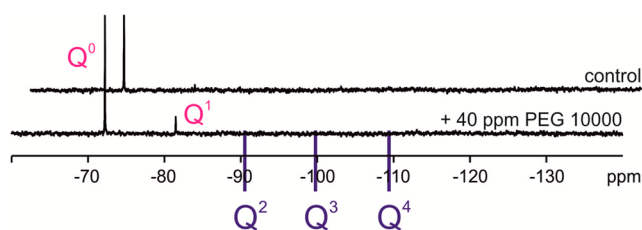


Figure 3. Liquid-state ^{29}Si NMR spectra of a solution containing $\text{Na}_2^{29}\text{SiO}_3$ (500 ppm as SiO_2) without PEG addition (control) and with 40 ppm ($4 \mu\text{M}$) of PEG 10000 (as indicated), measured 2 days after preparation at pH 7.0. Characteristic literature values for the isotropic chemical shifts of Q^2 , Q^3 , and Q^4 in silica gel⁸⁶ are indicated.

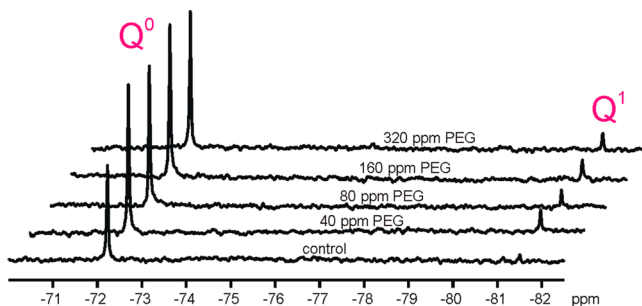


Figure 4. Liquid-state ^{29}Si NMR spectra of a solution containing 500 ppm SiO_2 and various concentrations of PEG 10000 (as indicated) 2 days after preparation at pH 7.0. The spectral region characteristic for Q^0 and Q^1 is shown, which gives rise to well-resolved lines in contrast to higher oligomers in the form of colloidal silica⁶⁶ (cf., also Figure 3 and Supporting Information Figure S-4).

Table 1. Concentrations of Q^0 and Q^1 Species Determined by ^{29}Si NMR and the Silicomolybdate (Molybdenum Blue) Test Measured 2 days after Preparation

sample	Si species (ppm) ^{29}Si NMR			molybdate-reactive silica
	Q^0	Q^1	$\text{Q}^0 + \text{Q}^1$	
control	199	15	214	184
PEG 10000 (40 ppm, $4 \mu\text{M}$)	345	56	401	374

signals characteristic for monosilicic acid (Q^0) and disilicic acid (Q^1) can be observed. Because the spectra were measured under identical conditions, the signal intensities observed in the spectra of Figure 3 can be directly compared and, furthermore, be used to measure absolute values for concentrations by comparing the intensities, that is, signal areas, with that of a reference sample. The sum of the signal intensities of these two species represents the molybdate-reactive silica present in the sample. First, it is obvious that both signals, Q^0 and Q^1 , are more intense in the PEG 10000-containing sample than in the control. The resulting concentrations are given in Table 1.

Obviously, the presence of 40 ppm PEG 10000 results in significant stabilization of both species, Q^0 and Q^1 . Note that the total Si concentrations ($\text{Q}^0 + \text{Q}^1$) determined by ^{29}Si NMR agree well with the concentrations measured by the molybdenum blue test within the experimental error of $\sim 10\%$ for the NMR data. This means the presence of PEG clearly results in a “supersaturated” solution, thus confirming the above-described results of the silicomolybdate test (see Figures 1 and 2). Furthermore, it is remarkable that the signals in the PEG-containing samples remain narrow. This, in turn, means

that mono- and disilicic acids contributing to the denoted signals in the spectra obviously remain very mobile, as revealed by the narrow line width. This further shows that the proposed hydrogen-bonding interactions can only lead to short-lived PEG–silicic acid “complexes” in rapid exchange with the free state of mono- and disilicic acids.

Inspection of Figure 3, furthermore, shows that the signals of higher oligomers (Q^2 , Q^3 , and Q^4) in colloidal silica are not detected. Note that the “background signal” caused by the NMR probe and the sample tube has been subtracted from the spectra shown in Figure 3 as described in the Supporting Information (Figure S-4). The absence of the signals due to higher oligomers is explained by the fact that these signals are strongly broadened and would only become visible at much higher Si concentrations as already described in detail previously.⁶⁶ Line-broadening is caused by an increasing degree of immobilization, which accompanies the oligomerization/polymerization process. Moreover, sedimentation and precipitation of heavier aggregates result in their “disappearance” from the active receiver coil volume in the NMR probe.

The influence of the concentration of PEG 10000 upon the observed supersaturation effect was also studied by liquid-state ^{29}Si NMR spectroscopy, Figure 4. Both Q^0 and Q^1 are stabilized by PEG 10000 at all studied concentrations (40–320 ppm). At 500 ppm SiO_2 , a concentration of 40 ppm PEG 10000 (4.0 μM) is already sufficient to obtain the full effect. Higher PEG concentrations do not provide further enhancement.

Formation of silica-containing precipitates is observed in the presence of PEG polymers, in contrast to the gel formed in the absence of PEGs. These precipitates exhibit degrees of silica polycondensation similar to those found in the “control” gel as can be seen from the ^{29}Si MAS NMR spectra (Supporting Information, Figure S-5). However, the 2D HETCOR spectra (Supporting Information, Figure S-6) show different ^1H NMR signal shifts. The signal due to H-bonded $\text{SiOH}/\text{H}_2\text{O}$ is found at 6 ppm in precipitates from the “control” solution. This signal is shifted to ca. 7 ppm in the PEG-containing precipitate. Moreover, the relative contribution of the H-bonded species to the total CP intensity is larger in the PEG-containing precipitate as compared to the PEG-free control. This indicates⁸⁷ the formation of slightly stronger H-bonds than in the “control” solid sample, most likely between PEG and the silica species/particles. The presence of PEG in these precipitates is unequivocally proven by ^{13}C CP MAS NMR spectroscopy of the precipitates (Supporting Information, Figure S-7). Note that the ^{13}C signal of PEG significantly shifts and is much narrower in the precipitates as compared to the pristine PEG, indicating that PEG molecules are embedded into the silica or at least bound to its surface (or both), and not phase separated. FT-IR spectra provide further corroborative proof for the interaction of precipitated silica particles and PEG. H-bonding between silanol and ethereal O ($-\text{CH}_2-\text{CH}_2-\text{O}-$) should perturb the band positions attributed to vibrations involving the O atom. The FT-IR spectra of PEG 10000 (Supporting Information, Figures S-8–S-11) show intense peaks assigned to C–O-related vibrations at 1148 cm^{-1} (C–O stretch) and 1093 cm^{-1} (C–O–C stretch). The former splits into two bands at 1152 and 1145 cm^{-1} , and the latter shifts to 1089 cm^{-1} . Such shifts have been attributed to silica–PEG H-bonding interactions⁸⁸ corroborating the proposed presence of PEG–silica hydrogen bonding. The silica–PEG precipitates are amorphous, with the PEG molecules

showing no long-range order as evidenced by X-ray powder diffraction (see Supporting Information, Figure S-26).

Amorphous silica precipitates were collected in the presence of PEGs after 8 h and 3 days, and studied by SEM, Figure 5

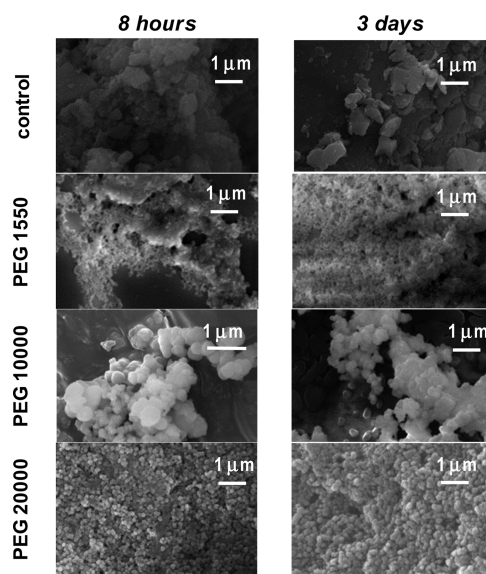


Figure 5. Selected SEM images of silica precipitates (formed at pH 7.0) in the presence of PEG 10000. The “control” images were taken on a dried gel sample.

(see Figure S-27 in the Supporting Information for all SEM images). A general observation is the tendency for particle aggregation upon prolonged $\text{Si}(\text{OH})_4$ exposure to PEG. Second, silica particles seem to possess the common spherical morphology. Finally, with increasing PEG MW, and hence chain elongation, there is a decrease in particle size and more pronounced aggregation, obviously due to PEG association with the silica particles as corroborated by the NMR and FT-IR results. A systematic enhancement of aggregation upon PEG chain length increase was also observed by Vong et al.⁸⁹

Figure 6 shows a magnification of silica spherical particles isolated in the presence of PEG 20000 after 24 h. It appears

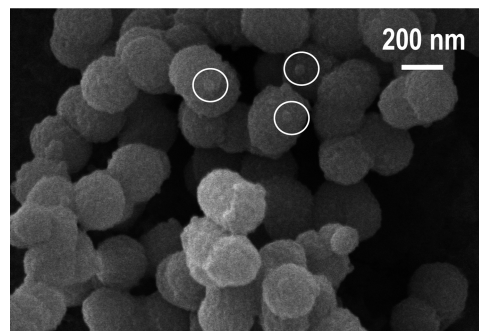


Figure 6. SEM image (magnification) of silica precipitates (formed at pH 7.0) in the presence of PEG 20000. Primary particles are indicated with circles.

that the surface of the particles is rough, an indication that even these “larger” particles are not the primary ones, but are rather dense aggregates of much smaller (<5 nm) primary particles, such as the ones indicated in Figure 6. PEG-induced silica particle aggregation has been reported by Killmann and

Winter⁹⁰ for PEGs of MW > 600 and by Rubio and Kitchener⁹¹ for polyethylene oxides with MWs of millions.

Interactions of PEG or PEG-containing polymers with silica particles have been reported previously. Kind et al. have invoked the presence of PEG in di- or tri-block polyethyleneimine/PEG polymers and its ability to form H-bonds with the surface silanols as a contribution to silica colloidal stability.⁹² Another study suggested that PEG 400 formed helical structures on fumed silica surfaces.⁹³ Parida et al. showed that partial PEG adsorption on silica, with a portion of the polymer protruding into the solution, is possible.⁹⁴ Other moieties grafted on polymers have also been reported to form H-bonds with silanols. Annenkov and co-workers have reported such interactions for the imidazole group on 1-vinylimidazole-acrylate copolymers⁵⁹ and for poly(1-vinylimidazole).^{95,96} Similar observations were noted for benzimidazole-based polymers.⁹⁷

The results described herein leave little doubt that the major mechanism for mono- and disilicic acid stabilization is H-bonding between silanol groups (H-donor) and the polyether O's (H-acceptor). A short discussion on silica-PEG hydrogen-bonding interactions is warranted at this point. Because of the pronounced instability of monosilicic acid (and disilicic acid as well), it is difficult to directly study its hydrogen-bonding behavior, especially in aqueous solutions. This is reflected by the total lack of corresponding literature. Such polyether-silicic acid hydrogen-bonding interactions are short-lived and undergo rapid exchange processes, which makes them hardly accessible by experimental techniques.

However, several researchers have studied organosilane molecules that possess one (or more) silanol (–Si–OH) groups. We mention some representative examples here. X-ray crystallographic analysis revealed that (tBuMe₂Si)₃SiOH forms a dimeric structure with hydrogen bonding between the silanol groups.⁹⁸ A review provides a section on hydrogen bonding of silanol-containing molecules.⁹⁹ The authors cite a variety of references describing the hydrogen-bonding behavior of silanols (in organosilane molecules) by FT-IR, including crystallographic proof for the existence of hydrogen bonds involving the silanol group. Another paper presented theoretical and FT-IR investigations on the hydrogen bonds between silanols and ethers or ketones such as dimethyl ether, diethyl ether, tetrahydrofuran, etc.¹⁰⁰

However, our main concern was to locate publications that offer appropriate information on hydrogen bonds between the silanol group and polyether oxygen atoms (ideally from PEG-like molecules). All available literature concerns such H-bonding interactions in the solid state (studied by appropriate solid-state techniques). Papisov et al.^{101,102} described precipitates formed by silicic acid in benzene solutions in the presence of poly(ethylene glycol) as a macromolecular matrix using tetra(acetoxysilane) as the “Si” source. On the basis of the provided experimental data, the authors propose two hydrogen-bonding schemes, inter- and intrachain, between the silanol groups and the PEG ethereal oxygens. Other authors have studied the ability of PEG polymers to form H-bonds with water proving the ability of PEGs to form such H-bonds.¹⁰³ Vong et al.⁸⁹ prepared and characterized PEG-modified silica gels prepared from tetraethyl orthosilicate (TEOS). The presence of PEG-silica hydrogen bonds was suggested. Koksall et al.¹⁰⁴ used polyethylene oxides (PEGs of high molecular weight) to flocculate sodium Kaolinite (an aluminosilicate) and suggested hydrogen bonding between PEG ethereal oxygens

and surface hydroxyl groups as being responsible for the flocculation activity. Silica-PEG sonogels were prepared via the sol-gel route starting with TEOS.¹⁰⁵ Hydrogen bonding was invoked after hydrolysis of TEOS. Direct measurements were made to quantify the molecular forces between streptavidin and lipid bilayers displaying grafted PEG 2000.¹⁰⁶ These measurements provided direct evidence for the formation of relatively strong attractive forces between PEG and the protein. These forces were assigned to the ability of PEG to form hydrogen bonds. Although this Article is not related to PEG-silanol hydrogen bonding, it provides experimental proof that PEG is able to form strong hydrogen bonds with appropriate H-donors. Kuraoka et al.¹⁰⁷ utilized PEG to form silica-PEG hybrid composites using TEOS as the Si source. PEG-silica hydrogen bonding was proposed as the main reason for the formation of these composites. FT-IR evidence was provided. Rubio and Kichener⁹¹ have discussed that the study of ethyleneoxide-silica hydrogen bonding by FT-IR spectroscopy in the aqueous state is very difficult. Therefore, PEO-silica precipitates (PEO = polyethylene oxide) were studied instead, and proof for hydrogen bonding was given for the solid materials. Malmsten et al.¹⁰⁸ studied the adsorption of polyethylene and polypropylene oxide (EO/PO) block copolymers on silica and suggested the existence of hydrogen bonds. The capability of PEGs to form hydrogen bonds with the silica surface was also proposed in another report.¹⁰⁹ Detailed considerations concerning the adsorption of organic molecules on silica surfaces are summarized in a recent review article.¹¹⁰ Strong PEG-silica interactions allowed the preparation of PEG-silica hybrids starting from sodium silicate.¹¹¹ Band shifts observed in the FT-IR spectra were interpreted as proof for these interactions. This is in line with the NMR and FT-IR investigations carried out on our PEG-silica precipitates (see above). In summary, there is sufficient convincing experimental proof for the presence of hydrogen bonding between silanol groups and PEG-like molecules, at least in the solid state.

Iler described an interesting method to quantify the hydrogen-bond formation ability of certain polar organic molecules (alcohols, ketones, amides, amines, carboxylic acids, and, importantly, PEGs).^{77,112,113} Briefly, the methodology is based on the ability of these molecules, coined “hydrogen-bonding agents”, to inhibit the precipitation of polysilicic acid with gelatin, by forming hydrogen bonds with polysilicic acid. A group of short-chain PEGs was tested (with 1, 2, 3, 5, and 8 ether moieties) against a “standard” (dimethoxytetraethylene glycol). Iler came up with certain values for the “molar effectiveness” and “effectiveness per ether group”. The latter are shown in the inset of Figure 7. We used these values to construct a simple mathematical equation:

$$y = 0.2126x^2 + 0.0797x + 14.538 \quad (R^2 = 0.999)$$

that relates “effectiveness per ether group” of our tested PEGs with the number of ether groups (or ethylene units) per PEG chain. The calculated “effectiveness per ether group” is plotted in Figure 7. The idea proposed by Iler is that the effectiveness of ether moieties in hydrogen bonding is not additive. When connected to each other in a single polymer chain, their ability to form hydrogen bonds exponentially increases with the number of ethylene oxide units. Hence, PEG 20000 has roughly 10 times more ethylene oxide units than does PEG 2000, but it is ~100 times more effective in hydrogen bonding.

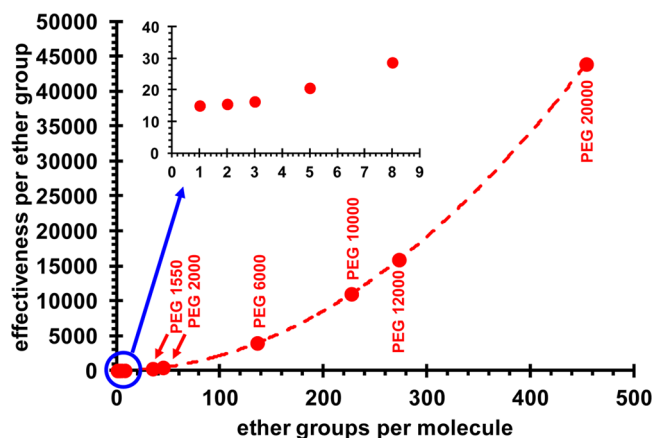


Figure 7. Calculated dependence of hydrogen-bonding effectiveness per ether group on the number of ether groups per PEG molecule. The inset includes experimental values published by Iler.¹¹²

To shed further light onto the stabilization mechanism, the following discussion is put forth. We will discuss PEG 10000, referring to Figure 1, but a similar analysis can be worked out for each PEG (the full analysis can be found in the Supporting Information, Figures S-12–S-25). We will only include silicic acid in this discussion, to keep the analysis simple. In other words, we will assume that all of the molybdate-reactive silica consists of monosilicic acid, exclusively. Each O atom of the PEG backbone can (theoretically) form a maximum of two H-bonds. In other words, each PEG 10000 molecule (containing 227 O atoms) can stabilize a maximum number of 454 molecules of $\text{Si}(\text{OH})_4$. By considering that 60 ppm ($6 \mu\text{M}$) of PEG 10000 stabilizes 177 ppm molybdate-reactive silica ($370 - 193 = 177$ ppm or 2.94 mM silicic acid above the “control” level) after 8 h, the molar ratio of Si:PEG is 490. This corresponds to virtually 100% loading of the PEG 10000 with $\text{Si}(\text{OH})_4$ molecules, while the PEG fully utilizes all of its O’s to stabilize $\text{Si}(\text{OH})_4$ at that concentration. By repeating the analysis for other PEGs at various concentrations, % loadings can be obtained for each experiment (see Supporting Information, Figures S-12–S-25). On the basis of this analysis, Figure 8 shows the dependence of Si:O ratio on PEG 10000

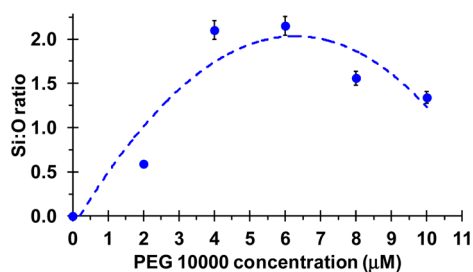


Figure 8. Dependence of the Si:O ratio (Si is silicic acid and O is the PEG oxygen) on PEG 10000 concentration. Data from 8-h experiments were used. The line is added to guide the eye.

concentration. The maximum value of the Si:O ratio should not exceed the theoretical maximum of 2 in agreement with our observations.

Finally, Figure 9 shows the dependence of molybdate-reactive silica on PEG MW at constant PEG mass content in solution (100 ppm). The implication is significant. Solutions containing the same PEG mass (100 ppm) exhibit substantially

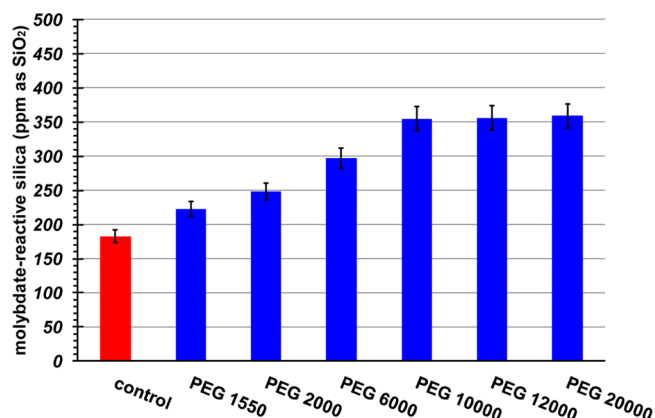


Figure 9. Dependence of molybdate-reactive silica on PEG MW at constant PEG mass content (100 ppm). The graph refers to 8-h data.

different stabilization activity, with the higher MWs being more effective. This is a strong indication that structural features of the PEG backbone, such as the number of repeating $-\text{CH}_2\text{CH}_2\text{O}-$ units connected in the same polymer chain, are profoundly important. The results of Figure 8, importantly, confirm that the increase of solution viscosity (if any) is not the primary mechanism of $\text{Si}(\text{OH})_4$ stabilization. In the case of a pure viscosity effect, the amount of molybdate-reactive silica should not level off beyond MW 10000 because the viscosity increases with the polymer chain length.

CONCLUSION

In summary, our present work provides the first evidence that polycondensation of $\text{Si}(\text{OH})_4$ is substantially retarded by uncharged macromolecules possessing ether moieties as interaction sites. Monosilicic and disilicic acids stabilization is profoundly dependent on PEG chain-length (and hence MW) and concentration. SEM also provides evidence of PEGs affecting silica particle morphology and aggregation. Finally, our work may spark alternative ways to envision “Si” stabilization (prior to biosilica formation) in diatoms and other organisms, by invoking possible roles of uncharged moieties among the rapidly growing family of biomacromolecules known to be important for biosilica synthesis.^{35–44,114–118}

ASSOCIATED CONTENT

Supporting Information

Full details on stabilization analyses, NMR, FT-IR, SEM studies, and relevant instrumentation. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

demadis@chemistry.uoc.gr

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

E.B. acknowledges support by the Deutsche Forschungsgemeinschaft (BR 1278/12-3 and 24-1). K.D.D. thanks the EU for funding the Research Program SILICAMPS-153, under the ERA.NET-RUS Pilot Joint Call for Collaborative S&T projects.

REFERENCES

- (1) Bidle, K. D.; Manganelli, M.; Azam, F. *Science* **2002**, *298*, 1980.

- (2) Struyf, E.; Smis, A.; Van Damme, S.; Meire, P.; Conley, D. J. *Silicon* **2009**, *1*, 207.
- (3) Street-Perrott, F. A.; Barker, P. A. *Earth Surf. Processes Landforms* **2008**, *33*, 1436.
- (4) Round, F.; Crawford, R.; Mann, D. *The Diatoms*; Cambridge University Press: Cambridge, 1990.
- (5) Seckbach, J.; Kociolek, P. *The Diatom World*; Springer: Dordrecht, 2011.
- (6) Compton, J. C. *Diatoms: Ecology and Life Cycle*; Nova Science Publishers, Inc.: New York, 2013.
- (7) Smol, J. P.; Stoermer, E. F. *The Diatoms: Applications for the Environmental and Earth Sciences*; Cambridge University Press: Cambridge, 2010.
- (8) Lewin, J. C. *Plant Physiol.* **1955**, *30*, 129.
- (9) Del Amo, Y.; Brzezinski, M. A. *J. Phycol.* **1999**, *35*, 1162.
- (10) Hildebrand, M.; Volcani, B. E.; Gassman, W.; Schroeder, J. I. *Nature* **1999**, *385*, 688.
- (11) Curnow, P.; Senior, L.; Knight, M. J.; Thamtrakoln, K.; Hildebrand, M.; Booth, P. J. *Biochemistry* **2012**, *51*, 3776.
- (12) Annenkov, V. V.; Basharina, T. N.; Danilovtseva, E. N.; Grachev, M. A. *Protoplasma* **2013**, *250*, 1147.
- (13) Dugdale, R. C.; Wilkerson, F. P. *Sci. Mar.* **2001**, *65*, 141.
- (14) Treguer, P. J.; De La Rocha, C. L. In *Annual Review of Marine Science*; Carlson, C. A., Giovannoni, S. J., Eds.; Annual Reviews Scientific Publishers: Palo Alto, CA, 2013; Vol. 5, p 477.
- (15) Treguer, P. J.; Nelson, D. M.; Van Bennekom, A. J.; DeMaster, D. J.; Leynaert, A.; Queguiner, B. *Science* **1995**, *268*, 375.
- (16) Thamtrakoln, K.; Hildebrand, M. *Eukaryotic Cell* **2007**, *6*, 271.
- (17) Thamtrakoln, K.; Hildebrand, M. *Plant Physiol.* **2008**, *146*, 1397.
- (18) Hildebrand, M. In *Biom mineralization*; Bäuerlein, E., Ed.; Wiley: Weinheim, 2000; p 170.
- (19) Sumper, M.; Brunner, E. *ChemBioChem* **2008**, *9*, 1187.
- (20) Hazelaar, S.; van der Strate, H. J.; Gieskes, W. W. C.; Vrieling, E. *G. J. Phycol.* **2005**, *41*, 354.
- (21) Vrieling, E. G.; Gieskes, W. W. C.; Beelen, T. P. M. *J. Phycol.* **1999**, *35*, 548.
- (22) Hildebrand, M. In *Biom mineralization: Progress in Biology, Molecular Biology and Application*; Bäuerlein, E., Ed.; Wiley-VCH: Weinheim, 2005; p 159.
- (23) Brasser, H. J.; van der Strate, H. J.; Gieskes, W. W. C.; Krijger, G. C.; Vrieling, E. G.; Wolterbeek, H. T. *Silicon* **2012**, *4*, 39.
- (24) Werner, D. *Arch. Mikrobiol.* **1966**, *55*, 278.
- (25) Chisholm, S. W.; Azam, F.; Eppley, R. W. *Limnol. Oceanogr.* **1978**, *23*, 518.
- (26) Martin-Jézéquel, V.; Hildebrand, M.; Brzezinski, M. A. *J. Phycol.* **2000**, *36*, 821.
- (27) Martin-Jézéquel, V.; Lopez, P. *Prog. Mol. Subcell. Biol.* **2003**, *33*, 99.
- (28) Pickett-Heaps, J. D.; Schmid, A. M. M.; Edgar, L. A. *Prog. Phycol. Res.* **1990**, *7*, 1.
- (29) Ketsetzi, A.; Stathoulopoulou, A.; Demadis, K. D. *Desalination* **2008**, *223*, 487.
- (30) Demadis, K. D. In *Science and Technology of Industrial Water Treatment*; Amjad, Z., Ed.; CRC Press: London, 2010; p 179.
- (31) Demadis, K. D.; Pachis, K.; Ketsetzi, A.; Stathoulopoulou, A. *Adv. Colloid Interface Sci.* **2009**, *151*, 33.
- (32) Azam, F. *Planta* **1974**, *121*, 205.
- (33) Schmid, A.-M. M.; Schulz, D. *Protoplasma* **1979**, *100*, 267.
- (34) Schröder, H. C.; Natalio, F.; Shukoor, I.; Tremel, W.; Schloßmacher, U.; Wang, X.; Müller, W. E. G. *J. Struct. Biol.* **2007**, *159*, 325.
- (35) Kröger, N.; Deutzmann, R.; Sumper, M. *Science* **1999**, *286*, 1129.
- (36) Kröger, N.; Lorenz, S.; Brunner, E.; Sumper, M. *Science* **2002**, *298*, 584.
- (37) Wenzl, S.; Hett, R.; Richthammer, P.; Sumper, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 1729.
- (38) Richthammer, P.; Börmel, M.; Brunner, E.; van Pée, K.-H. *ChemBioChem* **2011**, *12*, 1362.
- (39) Scheffel, A.; Poulsen, N.; Shian, S.; Kröger, N. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 3175.
- (40) Kröger, N.; Deutzmann, R.; Bergsdorf, C.; Sumper, M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14133.
- (41) Sumper, M.; Brunner, E.; Lehmann, G. *FEBS Lett.* **2005**, *579*, 3765.
- (42) Chiovitti, A.; Harper, R. E.; Willis, A.; Bacic, A.; Mulvaney, P.; Wetherbee, R. *J. Phycol.* **2005**, *41*, 1154.
- (43) Brunner, E.; Richthammer, P.; Ehrlich, H.; Paasch, S.; Simon, P.; Ueberlein, S.; van Pée, K.-H. *Angew. Chem., Int. Ed.* **2009**, *48*, 9724.
- (44) Hedrich, R.; Machill, S.; Brunner, E. *Carbohydr. Res.* **2013**, *365*, 52.
- (45) Engelhardt, G.; Michel, D. *High Resolution Solid-State NMR of Silicates and Zeolites*; Wiley: Chichester, 1987.
- (46) Tesson, B.; Masse, S.; Laurent, G.; Maquet, J.; Livage, J.; Martin-Jézéquel, V.; Coradin, T. *Anal. Bioanal. Chem.* **2008**, *390*, 1889.
- (47) Gröger, C.; Lutz, K.; Brunner, E. *Prog. Nucl. Magn. Reson. Spectrosc.* **2009**, *54*, 54.
- (48) Gröger, C.; Sumper, M.; Brunner, E. *J. Struct. Biol.* **2008**, *161*, 55.
- (49) Brunner, E.; Gröger, C.; Lutz, K.; Richthammer, P.; Spinde, K.; Sumper, M. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 607.
- (50) Harris, R. K.; Knight, C. T. *G. J. Chem. Soc., Chem. Commun.* **1980**, 726.
- (51) Knight, C. T. G.; Kirkpatrick, R. J.; Oldfield, E. *J. Magn. Reson.* **1988**, *78*, 31.
- (52) Kinrade, S. D.; Gillson, A.-M. E.; Knight, C. T. *G. J. Chem. Soc., Dalton Trans.* **2002**, 307.
- (53) Kinrade, S. D.; DelNin, J. W.; Schach, A. S.; Sloan, T. A.; Wilson, K. L.; Knight, C. T. *G. Science* **1999**, *285*, 1542.
- (54) Kinrade, S. D.; Hamilton, R. J.; Schach, A. S.; Knight, C. T. *G. J. Chem. Soc., Dalton Trans.* **2001**, 961.
- (55) Kinrade, S. D.; Schach, A. S.; Hamilton, R. J.; Knight, C. T. *G. Chem. Commun.* **2001**, 1564.
- (56) Sumper, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 2251.
- (57) Sumper, M.; Lorenz, S.; Brunner, E. *Angew. Chem., Int. Ed.* **2003**, *42*, 5192.
- (58) Brunner, E.; Lutz, K.; Sumper, M. *Phys. Chem. Chem. Phys.* **2004**, *6*, 854.
- (59) Danilovtseva, E. N.; Pal'shin, V. A.; Likhoshway, Y. V.; Annenkov, V. V. *Adv. Sci. Lett.* **2011**, *4*, 616.
- (60) Demadis, K. D.; Neofotistou, E. *Chem. Mater.* **2007**, *19*, 581.
- (61) Neofotistou, E.; Demadis, K. D. *Colloids Surf., A* **2004**, *242*, 213.
- (62) Demadis, K. D. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 630.
- (63) Mavredaki, E.; Neofotistou, E.; Demadis, K. D. *Ind. Eng. Chem. Res.* **2005**, *44*, 7019.
- (64) Demadis, K. D.; Ketsetzi, A.; Pachis, K.; Ramos, V. M. *Biomacromolecules* **2008**, *9*, 3288.
- (65) Demadis, K. D.; Stathoulopoulou, A. *Ind. Eng. Chem. Res.* **2006**, *45*, 4436.
- (66) Spinde, K.; Pachis, K.; Antonakaki, I.; Brunner, E.; Demadis, K. D. *Chem. Mater.* **2011**, *23*, 4676.
- (67) Demadis, K. D.; Tsistraki, A.; Popa, A.; Ilia, G.; Visa, A. *RSC Adv.* **2012**, *2*, 631.
- (68) Patwardhan, S. V.; Emami, F. S.; Berry, R. J.; Jones, S. E.; Naik, R. R.; Deschaume, O.; Heinz, H.; Perry, C. C. *J. Am. Chem. Soc.* **2012**, *134*, 6244.
- (69) Puddu, V.; Perry, C. C. *ACS Nano* **2012**, *6*, 6356.
- (70) Vrieling, E. G.; Beelen, T. P. M.; van Santen, R. A.; Gieskes, W. W. C. *Angew. Chem., Int. Ed.* **2002**, *41*, 1543.
- (71) Zhang, J.; Han, F.; Wei, X.; Shui, L.; Gong, H.; Zhang, P. *Ind. Eng. Chem. Res.* **2010**, *49*, 2025.
- (72) Liufu, S.; Xiao, H.; Li, Y. *Powder Technol.* **2004**, *145*, 20.
- (73) Bahri, H.; Guveli, D. E. *Colloid Polym. Sci.* **1988**, *266*, 141.
- (74) Gonzalez-Tello, P.; Camacho, F.; Blazquez, G. *J. Chem. Eng. Data* **1994**, *39*, 611.

- (75) Józefczak, A.; Hornowski, T.; Skumiel, A.; Závishová, V.; Konecká, M.; Tomašovičová, N.; Timko, M.; Kopčanský, P.; Kelani, H. N. *Int. J. Thermophys.* **2012**, *33*, 640.
- (76) Icopini, G. A.; Brantley, S. L.; Heaney, P. J. *Geochim. Cosmochim. Acta* **2005**, *69*, 293.
- (77) Iler, R. K. *The Chemistry of Silica*; John Wiley & Sons: New York, 1979.
- (78) Suttle, C. A.; Price, N. M.; Harrison, P. J.; Thompson, P. A. *J. Physcol.* **1986**, *22*, 234.
- (79) Coradin, T.; Livage, J. *Colloids Surf., B* **2001**, *21*, 329.
- (80) Coradin, T.; Eglin, D.; Livage, J. *Spectroscopy* **2004**, *18*, 567.
- (81) Alexander, G. B. *J. Am. Chem. Soc.* **1953**, *75*, 5655.
- (82) Truesdale, V. W.; Smith, P. J.; Smith, C. J. *Analyst* **1979**, 897.
- (83) Truesdale, V. W.; Smith, C. J. *Analyst* **1975**, 797.
- (84) Truesdale, V. W.; Smith, C. J. *Analyst* **1975**, 203.
- (85) Eaton, A. D.; Clesceri, L. S.; Rice, E. W.; Greenberg, A. E.; Franson, M. H. *Silicon. Standard Methods for Examination of Water and Wastewater*; American Public Health Association: Washington, DC, 2005.
- (86) Maciel, G. E.; Sindorf, D. W. *J. Am. Chem. Soc.* **1980**, *102*, 7607.
- (87) Brunner, E.; Sternberg, U. *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *32*, 21.
- (88) Teberekidis, V. I.; Sigalas, M. P. *J. Mol. Struct. (THEOCHEM)* **2007**, *803*, 29.
- (89) Vong, M. S. W.; Bazin, N.; Sermon, E. A. *J. Sol-Gel Sci. Technol.* **1997**, *8*, 499.
- (90) Killmann, E.; Winter, K. *Angew. Makromol. Chem.* **1975**, *43*, 53.
- (91) Rubio, J.; Kitchener, J. A. *J. Colloid Interface Sci.* **1976**, *57*, 132.
- (92) Kind, L.; Shkilnyy, A.; Schlaad, H.; Meier, W.; Taubert, A. *Colloid Polym. Sci.* **2010**, *288*, 1645.
- (93) Derkaoui, N.; Said, S.; Grohens, Y.; Olier, R.; Privat, M. *Langmuir* **2007**, *23*, 6631.
- (94) Parida, S. K.; Dash, S.; Patel, S.; Mishra, B. K. *Adv. Colloid Interface Sci.* **2006**, *121*, 77.
- (95) Annenkov, V. V.; Danilovtseva, E. N.; Likhoshway, Y. V.; Patwardhan, S. V.; Perry, C. C. *J. Mater. Chem.* **2008**, *18*, 553.
- (96) Liang, M.-K.; Patwardhan, S. V.; Danilovtseva, E. N.; Annenkov, V. V.; Perry, C. C. *J. Mater. Res.* **2009**, *24*, 1700.
- (97) Ghosh, S.; Maity, S.; Jana, T. *J. Mater. Chem.* **2011**, *21*, 14897.
- (98) Unno, M.; Murakami, H.; Kagawa, S.; Matsumoto, H. *Silicon Chem.* **2005**, *3*, 195.
- (99) Chandrasekhar, V.; Boomishankar, R.; Nagendran, S. *Chem. Rev.* **2004**, *104*, 5847.
- (100) Reichstat, M. M.; Mioč, U. B.; Bogunovic, L. J.; Ribnikar, S. V. *J. Mol. Struct.* **1991**, *244*, 283.
- (101) Papisov, I. M.; Bolyachevskaya, K. I.; Litmanovich, A. A.; Matveenko, V. N.; Volchkova, I. L. *Eur. Polym. J.* **1999**, *35*, 2087.
- (102) Papisov, I. M.; Grushina, V. V.; Litmanovich, A. A.; Sapozhnikov, D. A.; Vygodskii, Y. S.; Gritskova, I. A. *Polym. Sci., Ser. A* **2010**, *52*, 842.
- (103) Xu, Q.; Mi, J.; Zhong, C. *J. Chem. Phys.* **2010**, *133*, 174104.
- (104) Koksai, E.; Ramachandran, R.; Somasundaran, P.; Maltesh, C. *Powder Technol.* **1990**, *62*, 253.
- (105) Jung, H. Y.; Gupta, R. K.; Seo, D. W.; Kim, Y. H.; Whang, C. M. *Bull. Korean Chem. Soc.* **2002**, *23*, 884.
- (106) Sheth, S. R.; Leckband, D. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8399.
- (107) Kuraoka, K.; Ueda, T.; Sato, M.; Okamoto, T.; Yazawa, T. *J. Mater. Sci.* **2005**, *40*, 3577.
- (108) Malmsten, M.; Linse, P.; Cosgrove, T. *Macromolecules* **1992**, *25*, 2474.
- (109) Parida, S. K.; Mishra, B. K. *Colloids Surf., A* **1998**, *134*, 249.
- (110) Parida, S. K.; Dash, S.; Patel, S.; Mishra, B. K. *Adv. Colloid Interface Sci.* **2006**, *121*, 77.
- (111) Elimelech, H.; Avnir, D. *Chem. Mater.* **2008**, *20*, 2224.
- (112) Iler, R. K. *J. Phys. Chem.* **1952**, *56*, 673.
- (113) Iler, R. K. In *Biochemistry of Silicon and Related Problems* (Nobel Foundation Symposia); Bendz, G., Lyndqvist, I., Eds.; Springer: New York, 1978.
- (114) Thamatrakoln, K.; Alverson, A. J.; Hildebrand, M. *J. Physcol.* **2006**, *42*, 822.
- (115) Shimizu, K.; Cha, J.; Stucky, G. D.; Morse, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6234.
- (116) Müller, W. E. G.; Rothenberger, M.; Boreiko, A.; Tremel, W.; Reiber, A.; Schröder, H. C. *Cell Tissue Res.* **2005**, *321*, 285.
- (117) Ehrlich, H. *Int. Geol. Rev.* **2010**, *52*, 661.
- (118) Ehrlich, H.; Deutzmann, R.; Brunner, E.; Cappellini, E.; Koon, H.; Solazzo, C.; Yang, Y.; Ashford, D.; Thomas-Oates, J.; Lubeck, M.; Baessmann, C.; Langrock, T.; Hoffmann, R.; Wörheide, G.; Reitner, J.; Simon, P.; Tsurkan, M.; Ereskovsky, A. V.; Kurek, D.; Bazhenov, V. V.; Hunoldt, S.; Mertig, M.; Vyalikh, D. V.; Molodtsov, S. L.; Kummer, K.; Worch, H.; Smetacek, V.; Collins, M. J. *Nat. Chem.* **2010**, *2*, 1084.