RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Induced Transformation of Amorphous Silica to Cristobalite on Bacterial Surface

Shuang-Hong Xue[1], Hao Xie[1,2,*], Hang Ping[2], Qi-Chang Li[1], Bao-Lian Su[2,3], Zheng-Yi Fu[2,*]

[1]School of Chemistry, Chemical Engineering and Life Science, Wuhan University of Technology, Wuhan, 430070, China

[2]State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, Wuhan, 430070, China

[3]Laboratory of Inorganic Materials Chemistry, University of Namur, B-5000 Namur, Belgium

***Corresponding author:** E-mail: h.xie@whut.edu.cn (H. Xie); zyfu@whut.edu.cn (Z.-Y. Fu)

Abstract

Extreme conditions such as high temperature and/or pressure are usually required for transformation of amorphous silica to crystalline polymorphs. In this article, we presented our results that amorphous silica can be deposited on bacterial surface and transformed to cristobalite at a relatively low temperature and ambient pressure. The phase transformation of amorphous silica to cristobalite under thermal treatment was investigated by a variety of methods including X-ray diffraction, electron microscopy, and Fourier transform infrared spectroscopy. Results show that amorphous silica on bacterial cell surface exhibits a direct phase transformation to cristobalite structure at a relatively low temperature (800^{o}C). Surface charge of bacterial cells does not affect the phase transformation. Three Gram negative bacteria and three Gram positive bacteria have been tested in the present study. All of these bacteria have been found to facilitate phase transition of amorphous silica into cristobalite. The observation of induced amorphous silica transformation on bacterial surface to cristobalite highlights the use of bacteria in the synthesis and the structure control of silica minerals.

.

Keywords

Amorphous silica; Bacterial surface; Cristobalite; Phase transformation

Introduction

Silica is with broad applications such as semiconductors, ceramics, high performance thin-film transistors, solar cells, and electro-optical devices. Quartz, cristobalite and tridymite are the main phases in silica bricks. Elastic properties of cristobalite at room temperature have been extensively studied for the auxetic behavior such as in fabrication of microelectronic devices.[1] However, applications of cristobalite are restricted due to extreme crystallization conditions such as high temperatures, high pressures, and/or the use of alkaline chemicals.[2-10] There have been many attempts in controlling crystallization of silica. Metal-induced crystallization (MIC) method for preparing polycrystalline silica from amorphous silica was developed based on observations that the phase transition temperature was significantly reduced in the presence of metals.[5,11-13] Template-induced crystallization (TIC) is another approach that improves crystallization conditions on different templates. For example, synthesis of polycrystalline cristobalite balls has been achieved by using colloid-imprinted carbon as a template.[14] Both MIC and TIC approaches require extensive interactions between precursors and inducers (or templates) for initiating and promoting crystallization. Biosilicification follows this manner that proteins act as inducers or templates for the silica crystallization. A recent report showed that the protein silicatein induced crystallization of amorphous silica at relatively low temperature and ambient pressure.[15] Roles of silicatein rely on interactions between silicatein and crystallization precursors of silica. Theoretically, it is possible to promote transformation of amorphous biosilica by substituting silicatein with organic molecules. These organic molecules are either chemically synthesized polymers or biologically produced recombinant proteins that are carefully designed to function as silicatein. However, these methods are rather complicated or technologically difficult, and limited by interacting specificities between inducers and precursors.

The present study aims to provide a straightforward approach to control and improve phase transformation of amorphous silica at relatively low temperature and ambient pressure. We propose to take advantages of bacterial cells for inducing transformation of amorphous silica to cristobalite. Deposition of amorphous silica on bacterial surface was facilitated by drying treatment. Multidisciplinary analysis tools such as Fourier transform infrared (FTIR) spectroscopy and electron microscopy were used to study the transformation of amorphous silica to cristobalite structure at ambient pressure and a relatively low temperature (800^{o}C).

Experimental

Bacterial cultivation and collection

Single bacterial colony was inoculated in 5 mL of Luria-Bertani (LB) medium, followed by shaking overnight at 37°C, 220 rpm. The cell suspension was inoculated in Luria-Bertani (LB) medium at a ratio of 1:50, followed by 220 rpm shaking at 37°C for 5 hours of cultivation until cell growth reached stationary phase. Cells were then harvested by centrifugation at 8000 g, 4°C for 5 minutes.

Amorphous silica deposition and transformation on bacterial cell surface

SiO_2 Ludox silica colloidal was diluted in PBS (8 g/L NaCl, 0.2 g/L KCl, 1.15 g/L $Na_2HPO_4 \cdot 7H_2O$ and 0.2 g/L KH_2PO_4, pH 7.3) at the final silica concentration of 2 mg/mL, followed by ultrasonic wave dispersion for 10 minutes. Deposition of silica on cells were initiated by mixing cells with the silica solution ($5x10^8$ cells/ml) and incubated at 37°C, 220 rpm for 12 hours, followed by centrifugation at 8,000 g, 4°C for 5 minutes and washed three times with distilled water to remove excess silica. Precipitates were resuspended in small volume of distilled water and subjected to overnight treatment of vacuum freeze-drying or heat drying at 60°C. Phase transition of bio-deposited silica was completed by heating at 800°C or other desired temperature in a muffle furnace for 360 minutes, followed by controlled cooling.

Encapsulation of cells with polyelectrolytes

Bacterial cells were coated with polyelectrolytes in a layer-by-layer (LbL) fashion by using alternating deposition of positively charged poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) complexes (noted as PAH/PAA) onto the surface of cells.[16, 17] The LbL procedure was started by coating cells with a positively charged PAH, since surface charge of the cells is negative. Polyelectrolytes were dissolved in PBS and mixed with same volume of bacterial cells, followed by incubation at room temperature for 15 minutes. Excess polyelectrolytes were washed and removed with PBS. Polycations and polyanions were sequentially coated on cells by repeating the procedure, resulting encapsulating cells with PAH/PAA multilayers (3/2) (3 layers of PAH and 2 layers of PAA).

Characterizations of silica transformation

X-ray powder diffraction (XRPD) patterns were acquired with a Bruker AXS D8 Advance diffractometer with Cu Kα (λ= 1.5418 Å) operating at 40 kV 20 mA. Samples were

mashed with an agate mortar and placed in a PP holder. Data were acquired from in 2θ range of 10^{o}-80^{o} at a rate of 0.05^{o} s^{-1}. FT-IR/ATR spectra were recorded with a Bruker Alpha spectrometer. Triplicated measurements were performed and samples were scanned for 64 times at a 4 cm^{-1} resolution in the region of 4000-450 cm^{-1} for each measurement. Surface morphology of samples was viewed and characterized with a scanning electron microscope (SEM S-4800).

Results and discussion

Effects of *E. coli* surface on silica transformation

Deposition of amorphous silica on *E. coli* surface was facilitated by drying overnight. The substance significantly changed from light yellow loose powder to white loose powder upon drying. Silica deposition on *E. coli* surface was investigated with SEM (Figure 1, Panel A) and Energy dispersive spectrometry (EDS) analysis (Figure S1, Panels A and A', see Supporting Information). SEM images revealed the change in *E. coli* cell surface roughness from smooth to rough. EDS data showed that Si (Kα 1.74 keV) and O (Kα 0.52 keV) are the major inorganic composition on cell surface deposits. These observations implied silica deposition on *E. coli* surface.

The phase transformation of amorphous silica to crystalline polymorphs is known to occur only at high temperature and/or pressure.[18, 19] It can be promoted by surface directing agent and/or with highly porous preordered silica (known as zeolites).[19] The first transformation from α-quartz to α-tridymite occurs at 870°C and from quartz to β-cristobalite at 1600°C.[20, 21] In the present study, the phase transformation of amorphous silica on *E. coli* surface was induced at 800^{o}C. This temperature is lower than typical temperature for phase transformation of amorphous silica. After heating at 800^{o}C for 360 minutes, SEM observed morphology changes of the deposited silica (Figure 1, Panel A'). Phase transformation of silica was examined with XRD and FTIR spectroscopy (Figure 1). The X-ray diffraction pattern (Figure 1, Panel B) of thermally treated samples showed characteristic peaks at 21.96^{o}, 28.44^{o}, 31.40^{o}, and 36.09^{o}. These peaks correspond to the spacing of the 101, 111, 102, and 200 crystal planes of SiO_2 α-cristobalite (JCPDS PDF No. 39-1425).[22] A X-ray diffraction peak was observed at 20.69° that is related to quartz SiO_2 (JCPDS PDF No. 46-1045). The phase transformation was also supported by HR-TEM for selected areas electron diffraction (SAED) patterns (Figure 1, inset of Panel B) and FTIR spectroscopy (Figure 1, Panel C). The cristobalite structure was identified in the FTIR spectrum with a peak at 620 cm^{-1} and a knee

at 1200 cm^{-1}.[23] Theses observations suggest that the transformation of amorphous silica to cristobalite on *E. coli* surface was achieved at relatively low temperature.

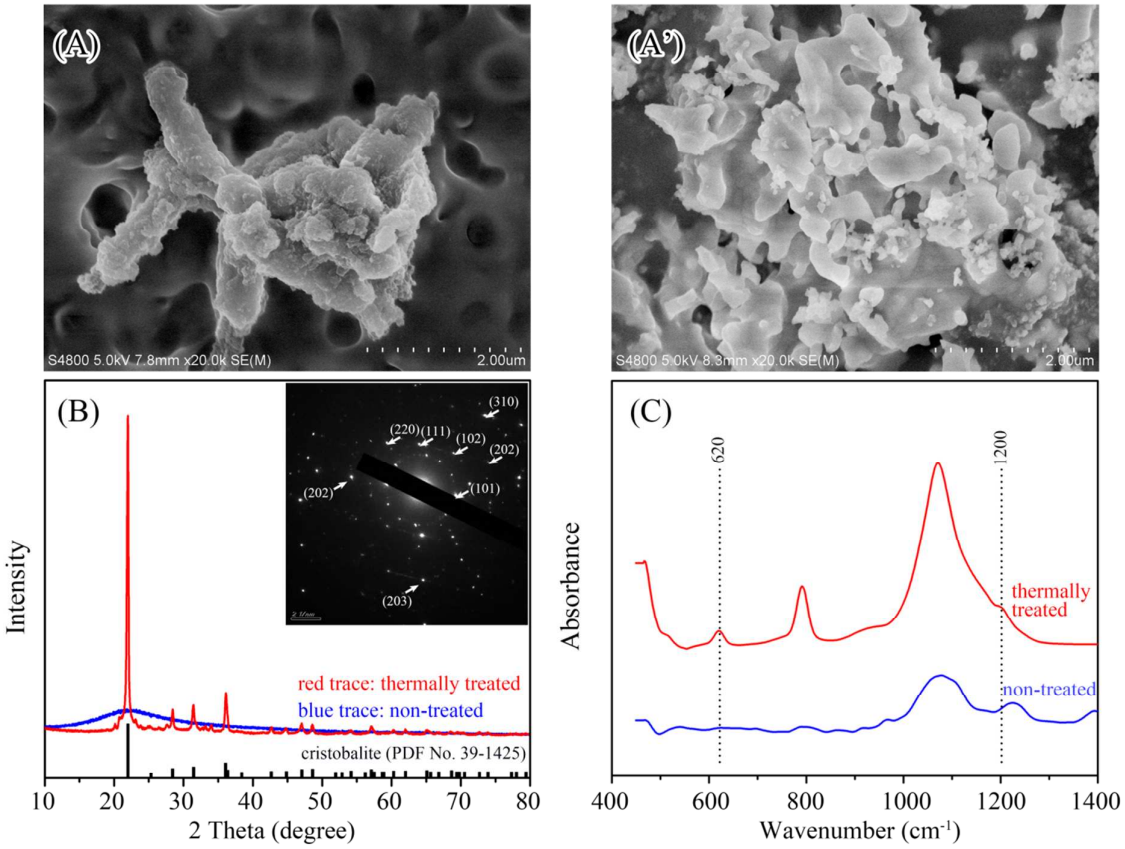

Figure 1. Transformation of silica on *E. coli* surface. Panel (A), SEM images of surface deposits on *E. coli*. Panel (A'), SEM images to show morphology changes of the deposited silica. Panel (B), XRD spectra of silica deposits before and after thermal treatment. Inset shows the HR-TEM SAED analysis of silica deposits after thermal treatment. Panel (C), FTIR spectra of silica deposits before and after thermal treatment.

We further investigated the critical phase transition temperature of amorphous silica on *E. coli* surface. When the sample was subjected to heating treatment at different temperatures for six hours, XRD spectra (Figure 2) shows the phase transformation of amorphous silica to cristobalite starts at 650°C. There is significant improvement of the crystallization when raising temperature up to 700°C. Complete crystallization is observed at 800°C. In subsequent experiments, 800°C was used as the temperature for heating treatment to induce phase transformation of amorphous silica to cristobalite.

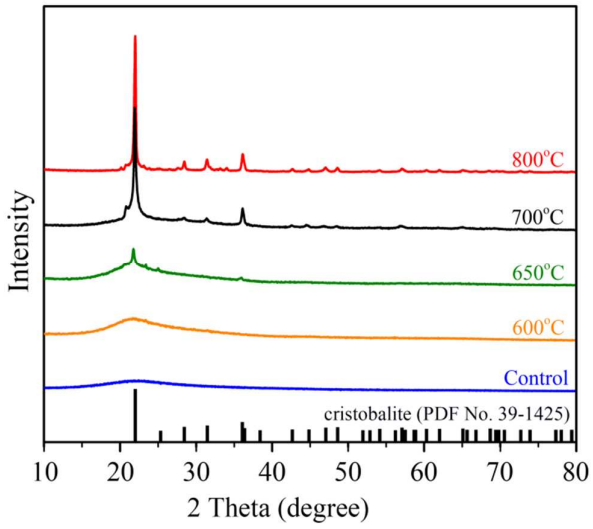

Figure 2. XRD spectra of silica deposits heated at different temperatures.

Effects of bacterial surface charge on inducing amorphous silica transformation

Bacteria can concentrate elements and act as nucleation sites for authigenic minerals.[24] Surface charges of bacteria and precursors may establish interactions between them through electrostatic adsorption. Studies on calcium carbonate suggested that polymorph selection is largely controlled by the surface charge density of amacrocyclic monolayer.[25,26] Therefore, we investigated effects of *E. coli* surface charge on inducing amorphous silica transformation. *E. coli* cells were coated with polyelectrolytes in a layer-by-layer (LbL) fashion with alternating deposition of PAH/PAA onto cell surface. Cell surface charge was then changed from negative to positive. Coated and uncoated *E. coli* cells were compared in inducing silica transformation. There is no significant difference of X-ray diffraction pattern between polyelectrolytes coated and uncoated *E. coli* cells (Figure 3). Both types of cells were observed in inducing transformation of amorphous silica to α-cristobalite. PAH alone or PAH and PAA together did not show influences in inducing silica transformation. These results suggested that *E. coli* surface charge did not contribute to phase transformation of amorphous silica.

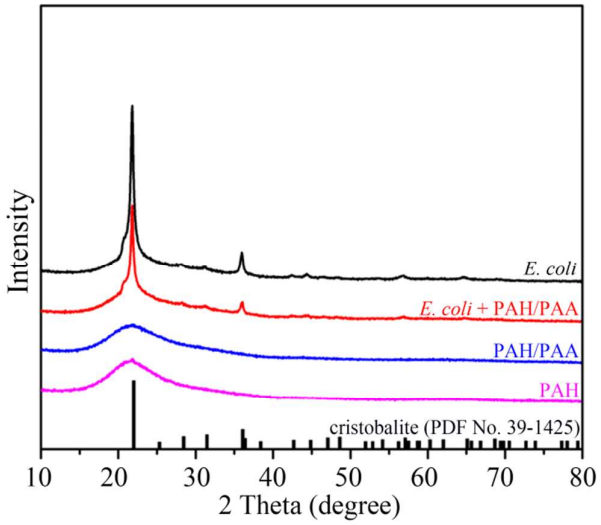

Figure 3. XRD spectra of silica deposited on *E. coli* (with or without polyelectrolytes coating) or polyelectrolytes after thermal treatment.

Silica transformation on different bacterial surface

Since surface charge of bacterial cells did not affect phase transformation of amorphous silica, we further investigated the specificity of bacterial strains with different surfaces in inducing amorphous silica transformation. Bacterial cells are distinct in their envelopes and fall into two major categories: a Gram-positive type and a Gram-negative type. In addition to the Gram-negative bacterium *E. coli*, five more representatives of the two types of bacteria including two Gram-negative bacteria (*Salmonella typhimurium* and *Proteus vulgaris*) and three Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*) were tested for their surfaces on amorphous silica transformation (Figure 4). Silica deposition on cell surface was observed for the two types of bacteria after drying treatment (Figure S1, see Supporting Information). SEM revealed morphology changes of bacteria after silica deposition and subsequent thermal treatment. Most bacterial cells collapsed after silica deposition promoting by drying treatment. After thermal treatment at 800^{o}C, porous structure with average pore size of 50-300 nm was generated from amorphous silica deposits on bacterial cells. Phase transformation of amorphous silica was proved by XRD. Characteristic peaks of α-cristobalite was identified at 21.83°, 28.33°, 31.29°, and 36.00° (JCPDS PDF No. 39-1425).[22] Phase transformation of amorphous silica was also demonstrated by FTIR spectra with a peak at 620 cm^{-1} and a knee at 1200 cm^{-1}. These peaks correspond to cristobalite.[23] These data proved that phase transformation of amorphous silica was achieved on surface of either Gram positive bacteria or Gram negative bacteria.

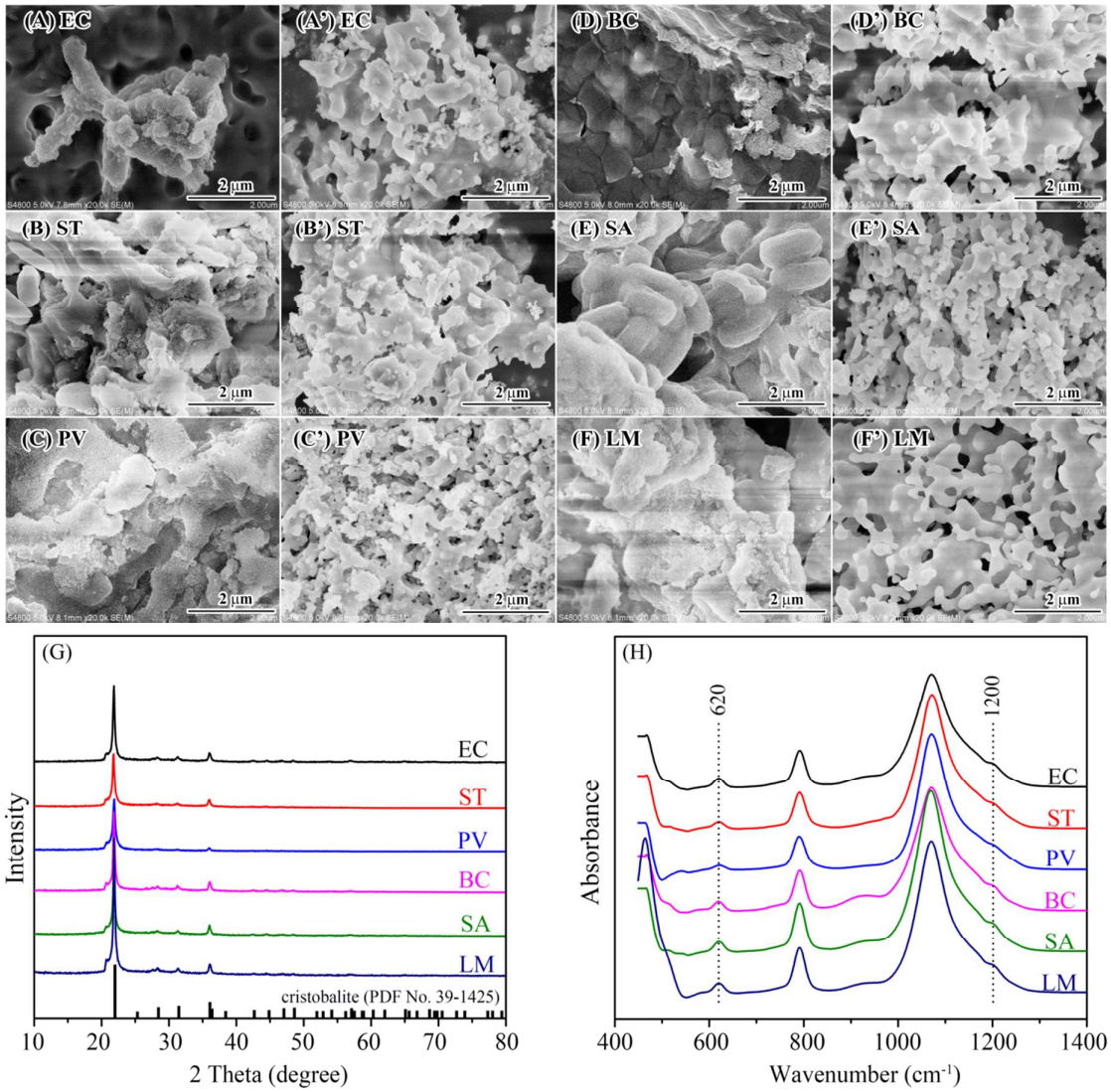

Figure 4. Transformation of silica on bacterial surface. Panels (A-F), SEM images of vacuum silica deposits on bacterial surface. Panels (A'-F'), SEM images to show morphology changes of the deposited silica after thermal transformation. Panel G, XRD spectra of transformed silica on bacterial surface. Panel H, FTIR spectra of transformed silica on bacterial surface. EC, *Escherichia coli*; ST, *Samlonella typhimurium*; PV, *Proteus vulgaris*; BC, *Bacillus cereus*; SA, *Staphylococcus aureus*; LM, *Listeria monocytogenes*.

Discussion

Phase transformation from amorphous precursors to crystals occurs in mineralization of many minerals such as $CaCO_3$, TiO_2 and SiO_2.[18,27-30] This process has been investigated in different biological environments or biomimetic conditions.[31-35] Various biological molecules have been tested for inducing phase transformation of amorphous precursors. Examples of these molecules are amino acids, polysaccharide, and proteins.[31-35] These

molecules interact with amorphous precursors and affects polymorph selections. In the present study, the surface of both Gram positive and negative bacteria is observed to promote transformation of amorphous silica. Although the two types of bacteria show different envelopes, both bacteria have phospholipids as the common component to form cell membranes. The phospholipids membranes play an important role in amorphous silica transformation. Studies on calcium carbonate suggested that polymorph selection is largely controlled by the surface charge density of amacrocyclic monolayer.[25,26] However, a recent report of calcium carbonate mineralization on phospholipid monolayer showed that the surface energy appears to be the ultimate determinant in polymorph selection.[36] Since we also observed that bacterial surface charge did not affect amorphous silica transformation, it is possible that the surface energy of phospholipid membranes promote the phase transformation of amorphous silica.

Conclusion

In summary, the present study showed that amorphous silica can be deposited on bacterial cell surface. Transformation of silica deposits on bacterial surface to cristobalite was achieved at relatively low temperature (800^{o}C) and ambient pressure. Surface charge of bacterial cells did not affect the phase transformation. Besides, phase transformation of amorphous silica can be induced on cell surface of both Gram positive and negative bacteria. The observation of induced silica transformation on bacterial surface opens a new avenue to take advantages of bacteria in the synthesis and the structure control of silica minerals.

Supporting Information

Electronic supplementary information (ESI) available.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (51161140399), the Ministry of Science and Technology of China (S2010GR0771), and the China Scholarship Council (CSC).

Reference

[1] M. Zeleny, J. Hegedus, A. S. Foster, D. A. Drabold, S. R. Elliott, R. M. Nieminen, *New J. Phys.*, 2012, **14**, 113029.

[2] M. Xu, S.W. Wang, X. X. Huang, J. K. Guo, *J. Mater. Res.*, 2005, **20**, 1943-1946.

[3] G. L. Humphrey, E. G. King, *J. Amer. Chem. Soc.*, 1952, **74**, 2041-2042.

[4] Y. Zhu, K. Yanagisawa, A. Onda, K. Kajiyoshi, *J. Mater. Sci.*, 2005, **40**, 3829-3831.

[5] P. Bruhns, R. X. Fischer, *Eur. J. Mineral.*, 2000, **12**, 615-624.

[6] X. M. Jiang, L. H. Bao, Y. S. Cheng, D. R. Dunphy, X. D. Li, J. Brinker, *Chem. Commun.*, 2012, **48**, 1293-1295.

[7] V. B. Prokopenko, L. S. Dubrovinsky, V. Dmitriev, H. P. Weber, *J. Alloy. Compd.* 2001, **327**, 87-95.

[8] L. S. Dubrovinsky, N. A. Dubrovinskaia, S. K. Saxena, F. Tutti, S. Rekhi, T. L. Bihan, *Chem. Phys. Lett.*, 2001, **333**, 264-270.

[9] Y. Tsuchida, T. Yagi, *Nature*, 1990, **347**, 267-269.

[10] F. Corwin, A. H. Herzog, G. E. Owen, R. G. Yalman, A. C. Swinnerton, *J. Amer. Chem. Soc.*, 1953, **75**, 3933-3934.

[11] W. Knaepen, C. Detavernier, R. L. V. Meirhaeghe, J. J. Sweet, C. Lavoie, *Thin Solid Films*, 2008, **516**, 4946-4952.

[12] D. Dimova-Malinovska, In *Nanostructured and Advanced Materials for Applications in Sensor, Optoelectronic and Photovoltaic Technology*, A. Vaseashta, D. Dimova-Malinovska, J. M. Marshall, Eds; Springer: Netherlands, 2005, 31-50

[13] A. Sarikov, *Appl. Phys. Lett.*, 2011, **99**, 143102.

[14] C. H. Kim, O. Teresa, *Bull. Korean Chem. Soc.*, 2011, **32**, 3483.

[15] I. Fuchs, Y. Aluma, M. Ilan, Y. Mastai, *J. Phys. Chem. B.*, 2014, **118**, 2104-2111.

[16] H. Park, S. H. Yang, J. Lee, E. H. Ko, D. Hong, I. S. Choi, *Adv. Mater.*, 2014, **26**, 2001-2010.

[17] K. Iler, *J. Coll. Interf. Sci.*, 1966, **21**, 569-594.

[18] P. Bettermann, F. Liebau, *Contrib. Mineral. Petrol.*, 1975, **53**, 25-36.

[19] A. Navrotsky, *Silica: Phys. Behav. Geochem. Mater. Appl.*, 1994, **29**, 309−329.

[20] R. Novakovic, S. M. Radic, M. M. Ristic, *Interceram*, 1986, **35**, 29-30.

[21] P. J. Heaney, C. T. Prewitt, G. V. Gibbs, *Silica: Physical. Behav. Geochem. Mater. Appl.*, 1994, **29**, 605.

[22] R. W. G. Wyckoff, *Am. J. Sci.*, 1925, **9**, 448-459.

[23] S. Shoval, M. Boudeulle, S. Yariv, I. Lapides, G. Panczer, *Opt. Mater.*, 2001, **16**, 319-327.

[24] K. Tazaki, H. Ishida, *Jour. Geol. Soc. Japan*, 1996, **102**, 866-878.

[25] M. Fricke, D. Volkmer, C. E. Krill, M. Kellermann, A. Hirsch, *Cryst. Growth Des.*, 2006, **6**, 1120-1123.

[26] M. Fricke, D. Volkmer, *Top. Curr. Chem.*, 2007, **270**, 1-41.

[27] J. W. Xiao, S. H. Yang, *CrystEngComm*, 2011, **13**, 6223-6230

[28] G. Madras, *Cryst. Growth Des.*, 2007, 7, 250-253

[29] H. Q. Xie, T. G. Xi, Q. H. Zhang, Q. R. Wu, *J. Mater. Sci. Technol.*, 2003, **19**, 463-466

[30] J. D. Rodriguez-Blanco, S. Shaw, L. G. Benning, *Geochim Cosmochim Acta*, 2008, **72(S1)**, A801-A801.

[31] S. W. Lee, Y. I. Kim, K. Lee, J. H. Bang, C. W. Jun, Y. N. Jang, *Mater. Trans.*, 2012, 53, 1732-1738.

[32] Y. Liu, Y. Cui, R. Guo, *Langmuir*, 2012, **28**, 6097-6105.

[33] H. Imai, N. Tochimoto, Y. Nishino, Y. Takezawa, Y. Oaki, *Cryst. Growth Des.*, 2012, **12**, 876-882.

[34] Y. Shchipunov, I. Postnova, *Colloid. Surface B*, 2009, **74**, 172-177.

[35] I. Postnova, E. Kozlova, S. Cherepanova, S. Tsybulya, A. Rempel, Y. Shchipunov, *RSC Adv.*, 2015, **5**, 8544-8551.

[36] J. Xiao, Z. Wang, Y. Tang, S. Yang, *Langmuir*, 2009, **26**, 4977-4983.