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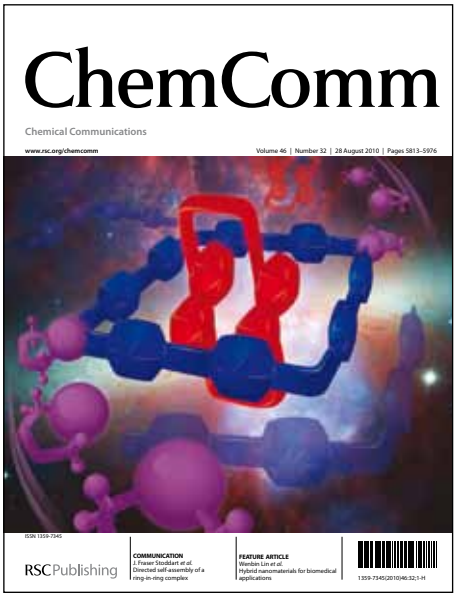

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Communications

Synthesis of transparent aminosilane-derived silica based networks for entrapment of sensitive materials

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A novel sol-gel synthesis route is reported which results in the formation of optically transparent silica based hydro- and xerogels from an aminosilane precursor in aqueous solutions. These materials can be used for entrapment of microalgae and of light-harvesting complex (LHC) samples.

Conventional sol-gel methods for preparation of silica materials are based on alkoxides such as tetraethylorthosilicate (TEOS) or tetramethylorthosilicate (TMOS). These precursors are hydrolyzed to form three-dimensional silica based networks.[1] Numerous modifications and applications of these sol-gel systems have been reported in literature. Organic components[2] and/or inorganic fillers[3] – among other approaches[4] – allow modifying the structures and properties of the obtained gels, glasses and hybrid materials. For optical applications and in particular for the entrapment and stabilization of light-harvesting components or living and photosynthetically active cells the silica materials should be as transparent for visible light as possible. Highly transparent hydro- and xerogels have frequently been obtained by the said sol-gel routes.[5] However, a serious disadvantage of TEOS, TMOS and other functionalized alkoxides as silica precursors for the immobilization of sensitive biomaterials including living cells is the fact that the reactivity of the precursors is relatively low. Therefore, the hydrolysis and condensation is catalyzed with acids or bases and sometimes elevated temperatures to reduce the gelation time.[1-5] Also, organic solvents are used and the by-product methanol and ethanol are toxic to cells. Another synthesis route to silica based networks is the use of sodium silicate involving high salt concentrations and high acid concentrations.[1,6] Alternative precursors lead to non-oxide materials such as aminosilanes for Si/N-based gels[7], carbodiimides for silicon carbonitrides and related materials.[8]

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However, these procedures generally require organic solvents in order to prevent hydrolysis and oxide formation.[9] These solvents are incompatible with sensitive biomaterials such as living microalgae.[10] Biocompatible biopolymer-based methods are gentle but suffer from stability and transparency.[11]

In the present work the above mentioned disadvantages associated with the use of Si-O precursors for the formation of silica based networks were overcome by using the precursor tetra(*n*-propylamino)silane $Si(NHPr)_4$. The rationale behind this approach is the higher reactivity of aminosilanes compared to the alkoxysilanes. "Silica based networks" is used as a generic term and includes two types of silica gels, a hydrogel and a glass-like xerogel, which are described in detail below. For the novel synthesis, the aminosilane precursor was first mixed with distilled water and stirred at room temperature forming an emulsion. The resulting particulate silica sol was then gelled by partial evaporation of the liquid phase. After gelation the aging process started, leading to glass-like xerogels. For optical measurements, hydrogels and glass-like xerogels were formed in open cuvettes. The gelling time for the initial aminosilane concentration of 25 % was 3 days after evaporation of 33 % of the liquid phase. Therefore, the synthesis was subsequently modified to speed up the procedure (Fig. 1). After sol formation for 6 h, the sol was poured onto glass petri dishes and converted to gel by evaporation with a constant airflow to about 30 % of the original volume.

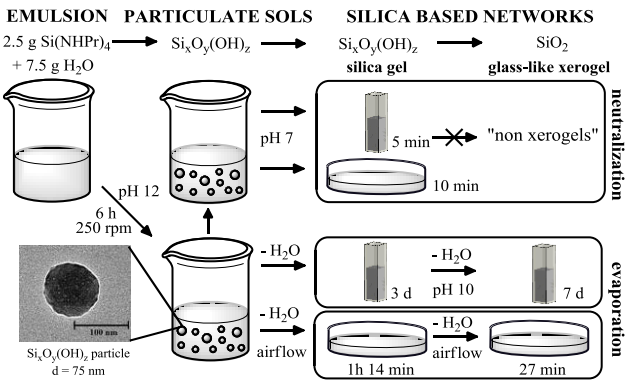

Fig. 1 Schematic route of the synthesis used for the preparation of aminosilane-derived silica based networks from particulate silica sols.

The gel then formed after 1 h 14 min and after further drying for 27 min the glass-like xerogel (Fig. 2B) formed. During the hydrolysis, the cleavage product *n*-propylamine was formed which lead to an increase of the pH of the sol from pH 7 to pH 12. Due to the evaporation of amine, the pH dropped to 10 during subsequent gel formation. Neutral pH values are desired for the development of biocompatible silica hydrogels. When the pH was adjusted to 7 after sol synthesis, i.e. the basic amine was transformed into the corresponding ammonium ion. It was observed that the gelling time was reduced to 10 min (25 % aminosilane concentration).

For optical applications these silica based networks must be transparent for visible light. Due the rapid reaction of the aminosilane precursor (1 min for hydrolysis), white precipitates were observed. Therefore, the question was clarified which concentrations yield optically transparent silica based networks. Thus, the influence of aminosilane precursor concentrations on the networks was studied with UV-Vis spectroscopy. The absorption was determined according to a standard DIN method and represents a measure of the transparency.[12] Absorption values below 0.06 indicated transparent silica based networks. As shown in Fig. 2A, the glass-like xerogels resulting from precursor concentration of 5 % to 25 % (pH 10) were transparent, but above 35 % the transparency decreased significantly. The biocompatible hydrogels retained transparency up to a concentration of 15 %, above this threshold the transparency strongly decreased.

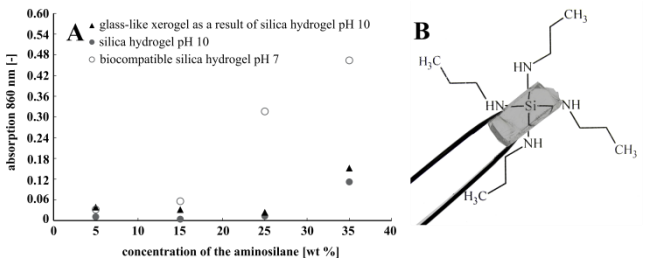

Fig. 2 Influence of the aminosilane precursor concentration on the silica based networks transparency (A) and a transparent glass-like xerogel (B). Transparency is illustrated by the clear visibility of the silicon atom when a gel block was placed over the lettering.

Furthermore, the resulting glass-like xerogels were studied with ^{29}Si and ^{13}C solid state nuclear magnetic resonance (NMR) spectroscopy. ^{29}Si solid state NMR spectra indicated a three-dimensional silica based network (chemical shift -109.7 ppm, fully condensed Q^4 units and chemical shift -99.4 ppm, Q^3 units).[13] The ^{13}C solid state NMR spectra showed that the gels contain *n*-propyl groups (chemical shift 11.6 ppm, 22.0 ppm and 24.0 ppm), which were assigned to *n*-propylamine entrapped inside the network and remaining Si-NHPr moieties (Fig. 3).

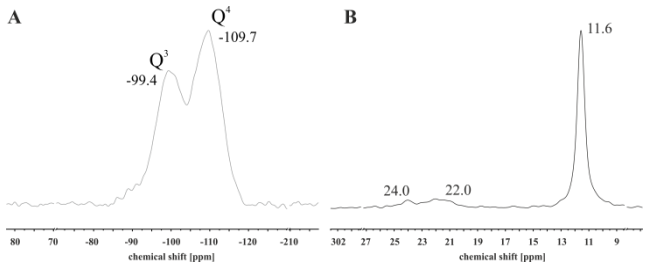

Fig. 3 ^{29}Si CP/MAS NMR spectrum (A) and ^{13}C CP/MAS NMR spectrum (B) of glass-like xerogel.

These results showed that the aminosilane precursor yields products analog to the known sol-gel process. The questions if microalgae and light-harvesting complex samples (LHC) can be entrapped in the biocompatible silica hydrogel were addressed. Generally, sol-gel processes based on alkoxysilanes have been used to immobilize biological material, but with limited success.[10,14] Problems associated with these approaches were the use of organic solvents to dissolve the precursor, strong acids and bases and toxic by-products formed, e.g. ethanol and methanol.

Chlamydomonas reinhardtii (*C. r.*) microalga wild type cells were used for entrapment first. Three different methods were applied to investigate if the entrapment procedure had any influence on the cell viability: microscopy to assess the cell morphology, chlorophyll fluorescence analysis to assess the activity of photosystem II and analysis of oxygen consumption and production to assess respiration and photosynthesis, respectively. Microscopic analysis (Fig. 4 A,B) revealed no morphological differences between free and entrapped cells. The influence on the photosynthetic activity of the cells was studied by measuring the maximum quantum yield of photosystem II (Φ PS II).[15] Before centrifugation, the Φ PS II of free cells was determined to be 0.73±0.003. This value dropped after centrifugation to 0.39±0.019, indicating a temporal decrease of the photosynthetic capacity. The Φ PS II dropped even further to 0.13±0.054 when the cells were entrapped, demonstrating a negative impact of the entrapment procedure. However, when cells were overlaid with algal growth media and stored in the dark for 2 h, the Φ PS II increased for the centrifuged entrapped cells to 0.43±0.014 and for the centrifuged free cells to 0.58±0.015, indicating that the photosynthetic activity was regenerated to some degree in both samples. Oxygen consumption[16] of free centrifugated cells was determined with a respiratory consumption of -8.04 nmol O_2 mL^{-1} min^{-1}. When measured 2 h after entrapment, the respiratory oxygen consumption dropped to -1.18 nmol O_2 mL^{-1} min^{-1} (Fig. 4C).

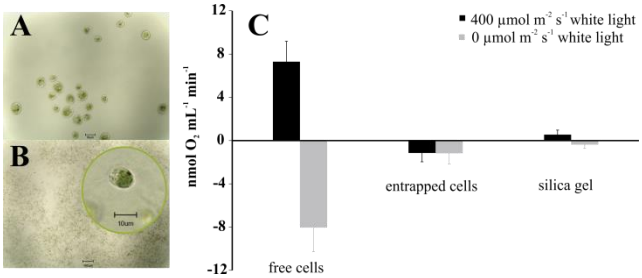

Fig. 4 Microscopic analysis of free cells (A), entrapped cells (B) and analysis of the oxygen consumption and production (C).

These data indicated that vital cells were present after entrapment, but vitality seemed to be reduced compared to free cells. It therefore can be concluded that *C. r.* cells can successfully be entrapped into this biocompatible silica hydrogel, but optimization seems desirable to reduce negative impacts on cell vitality and photosynthetic activity. The factors affecting cell physiology are not known yet, but it seems likely that *n*-propylamine at a concentration of 242 mg/L±13 could be responsible because it was reported to have herbicidal action.[17]

LHCs were chosen next for entrapment. UV-Vis spectra were used to investigate if entrapment had an influence on the stability. Free LHCs showed absorbance maxima at 430-470 nm (carote-

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noids) and 650-671 nm (chlorophyll a/b), corresponding to the different pigments bound to the complex. Entrapped LHCs showed no change of absorbance maxima after the gel formation (Fig. 5A). This indicates that the protein-pigment structure remained unchanged in the gel directly after entrapment. The influence of light exposure on the stability of LHCs at 671 nm showed that the maximal absorption of non-entrapped LHCs was reduced by 62 % after 2 h. After entrapment of the LHCs, the absorption was reduced by only 51 % after 2 h (Fig. 5B). These results indicate a stabilizing effect of the silica hydrogels.

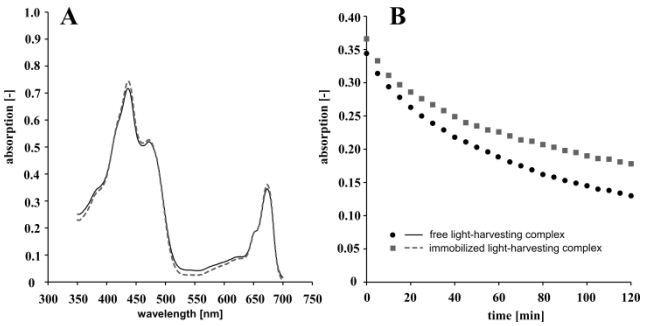

Fig. 5 The UV-Vis spectrum of free and entrapped LHCs (A) and the absorption as a function of time (B).

In conclusion, we showed that the $Si(NHPr)_4$ is a precursor for a facile and fast silica sol and gel formation at 25°C. Transparent hydrogels and glass-like xerogels are formed in aqueous solutions. The novel sol-gel synthesis route allows entrapping vital *C. r.* cells. Furthermore, we demonstrated that even sensitive isolated LHCs can be entrapped without loss of absorbance activity. These results indicate that the aminosilane-derived silica based networks can be suitable for coating, entrapment and/or stabilization of many other sensitive (bio)molecules and related optical and photochemical materials[18] in the future.

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Notes and references

Experimental data: All manipulations were performed in air except the synthesis of the aminosilane precursor, which was pepared under inert gas (N_2). The latter was synthesized according to a known procedure.[19] In brief, 500 mL *n*-hexane (which was dried and purified according to the standard method) and 36.3 g (0.614 mol) *n*-propylamine were stirred at 20°C and 13.1 g (0.077 mol) tetrachlorosilane were added dropwise. The resulting mixture was stirred at 20°C over night, then the *n*-propylamine hydrochloride was removed by Schlenk-filtration and washed with *n*-hexane. From the filtrate the solvent was removed under vacuum to yield a colourless oil. Solution NMR spectra were recorded on a Bruker DPX 400 spectrometer (^{1}H, 400.13 MHz; ^{29}Si, 79.49 MHz) in $CDCl_3$. CP/MAS NMR spectra were recorded on a Bruker Avance 400 Mz WB spectrometer using zirconia rotors with a 7 mm probehead (^{29}Si, 79.51 MHz; ^{13}C, 100.65 MHz). All chemical shifts are reported in ppm and refer to TMS. LHC were isolated from *C. r.* cells by 1 % ß-dodecyl maltoside detergent treatment.[20] For the entrapment of *C. r.* strain *cc124* was cultivated in tris acetat phosphate medium at 25°C. The cells were harvested by centrifugation at 500 × g for 1 min. The sol was added to the cell pellet. After 5 min a silica hydrogel is formed. Final cell concentration in the gel was ~1.2×10^7 cells/mL. The entrapment of the LHC was done with a mixture of 400 µL LHC und 3 mL of sol. UV-Vis spectroscopy was performed on a Thermo Scientific Genesys 10S. The Photosynthesis Yield Analyzer used in this study was a Walz Mini PAM instrument. Oxygen measurements were performed using a Clarktype oxygen electrode from HansaTech. The *n*-propylamine concentration was determined spectrophotometrically using "Dr. Lange cuvette test".

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