

Hybrid Polysaccharide–Silica Nanocomposites Prepared by the Sol–Gel Technique

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Received September 11, 2003. In Final Form: January 5, 2004

New monolithic nanocomposite silica biomaterials were synthesized on the basis of various natural polysaccharides and recently introduced completely water-soluble precursor tetrakis(2-hydroxyethyl) orthosilicate. The sol–gel processes were performed in aqueous solutions without the addition of organic solvents and catalysts. The silica polymerization was promoted by the polysaccharides through acceleration and catalytic effect on the processes. By introducing poly(vinyl alcohol) or poly(ethylene oxide) in the precursor solution, it was shown that the jellification took place in the case of the hydroxyl-containing polymer. Therefore, it was suggested that the catalysis was caused by a formation of hydrogen bonds between hydroxyl groups in macromolecules and products of precursor hydrolysis (silanols). It was also demonstrated that the polysaccharides radically changed the structure of biomaterials. In place of the cross-linked nanoparticles, there was a three-dimensional network from crossed or branched fibers and uncrossed spherical particles that filled the mesh space. The density of network, thickness of fibers, and properties of synthesized biomaterials depended on the polysaccharide type, charged degree of their macromolecule, and concentration. By varying these parameters, it was possible to manipulate the structural organization of hybrid polysaccharide–silica nanocomposites.

Introduction

The biomineralization processes leading to silica synthesis are widely spread in living nature. It is believed that they are regulated by proteins and polysaccharides.^{1–3} The role of proteins was confirmed in vitro by manipulation of the silica formation and structure by ones separated from diatoms and synthetic block copolypeptides in refs 4–6. It has been observed that polysaccharides, such as chitosan,⁷ alginate,⁸ and cyclodextrin,⁹ can also template silicate, but it was not examined in sufficient details.

The synthesis of silica nanocomposites by sol–gel processing is most commonly performed by using tetraethoxysilane (TEOS) or tetramethoxysilane (TMOS) as a precursor. Because they both do not dissolve enough in water, it is necessary to add an organic solvent.^{10,11} Its addition can have a denaturing effect and/or decrease the solubility of biopolymers, which sets limits on the possibility of the sol–gel technique to prepare biomaterials.¹² As an alternative to TEOS and TMOS, it was

suggested in ref 13 to use tetrakis(2-hydroxyethyl) orthosilicate (THEOS). The newly introduced precursor is completely water soluble, which obviates the need for organic solvent addition. As shown in the preliminary study, it is compatible with carrageenans.¹⁴ Furthermore, it has been successfully applied for the immobilization of enzymes.¹⁵

Here we report that THEOS is compatible with different natural polysaccharides. It was shown that they accelerated, catalyzed, and served as a template for silica generated in situ by the sol–gel processes, thus manipulating its synthesis as well as properties and structure of produced monolithic hybrid materials.

Experimental Section

Materials. The precursor tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was synthesized by using tetraethoxysilane (ABCOR, Germany) as described in detail in ref 16. *N*-[(Trimethoxysilyl)propyl]-*N,N,N*-trimethylammonium chloride was obtained from ABCOR. Kappa- and iota-carrageenans (both ca. 700 kDa), alginate, xanthan, and locust bean gum were purchased from Fluka. Lambda-carrageenan (1024 kDa, high degree of purification) was a gift from Hercules Copenhagen A/S (Denmark). Hydroxyethylcellulose (950 kDa) and its cationic derivatives (950 kDa) were obtained from Hoechst (Germany). Poly(vinyl alcohol) (hydrolyzed up to 88%) was supplied by Aldrich and poly(ethylene oxide) (Sedipur) by BASF. The polysaccharides and polymers were used as received. Their structural formula, molecular weight, type of charged groups, and some abbreviations are given in Table 1. Acetone was from Fluka. Distilled water was used to prepare solutions.

Hydrogel and Aerogel Preparation. The sol–gel processes were performed in accordance with the procedure developed in refs 14 and 15. A polysaccharide was initially dissolved in water

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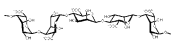
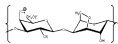

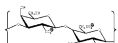
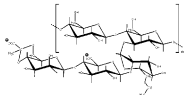

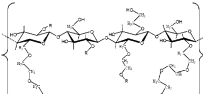
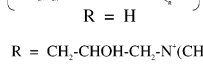
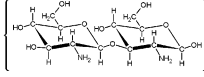


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Table 1. Polysaccharides Used to Prepare Hybrid Silica Nanocomposites

Polysaccharide/ Polymer	Structural formula	MW (kDa)	Charged groups	Hybrid hydrogel
Alginate		nd ^a	Anionic, carboxylic	Turbid, monolith
Kappa-carrageenan		700	Anionic, sulfate	Turbid, syneresis
Iota-carrageenan		700	Anionic, sulfate	Turbid, monolith
Lambda-carrageenan		1024	Anionic, sulfate	Turbid, monolith
Xanthan		nd	Anionic, carboxylic	Turbid, monolith
Locust bean gum		nd	Uncharged	Turbid, syneresis
Hydroxyethylcellulose (HEC)		950	Uncharged	Turbid, syneresis
Cationic derivative of hydroxyethylcellulose (cat-HEC)	 R = H R = CH ₂ -CHOH-CH ₂ -N ⁺ (CH ₃) ₃ Cl ⁻	950	Cationic, quaternary ammonium	Transparent, monolith
Chitosan		nd	Cationic, amino	Opalescent, monolith
Polyvinyl alcohol (PVA)		96	Uncharged	Turbid
Polyethyleneoxide (PEO)		>1.000	Uncharged	Not formed

^a Not determined.

and left for 1 day. After the addition of a weighed appropriate amount of precursor, the solution was thoroughly stirred and set aside at ambient temperature at least for 1 week. Because the gel could settle within a few minutes, this was the optimal period of time to complete the hydrogel formation. An examination of some samples after 9 months showed that their properties were not notably changed.

A silica nanocomposite in the absence of polysaccharide was synthesized as in ref 13 by dissolving THEOS, *N*-[(trimethoxysilyl)propyl]-*N,N,N*-trimethylammonium chloride, and sulfuric acid in water. To prepare an aerogel, at first the hydrogel was treated with acetone for substituting water and then the organic solvent removed by the hypercritical drying.

Experimental Methods. SEM micrographs were taken by a FE-SEM Leo 1530 electron microscope. The surface of samples was covered by a platinum layer ca. 1 μm in thickness.

Rheological measurements were performed with a Rotovisco RT 20 (Haake) stress-controlled rheometer. Measuring cells had cone and plate geometry. To determine the zero-shear viscosity of examined hydrogels, a creep regime was used.¹⁷ The yield stress was found from stress sweep measurements at an oscillation frequency of 1 Hz.¹⁸ The rheological measurements were performed at 25.0 ± 0.5 °C.

Results and Discussion

First, we examined whether polysaccharides (see Table 1) are compatible with THEOS. They were mixed together

in an aqueous solution. Any phase separation or precipitation was not observed. The solution also remained homogeneous after silica generation in situ. The sol–gel processes provided its jellification and formation of a monolithic hydrogel. This behavior was seen for all examined polysaccharides at the initial stages of processing.

Further maturation of hydrogels depended on the polysaccharide type. Samples containing uncharged biopolymers demonstrated a syneresis, i.e., a slow shrinkage of the gel volume accompanied by water release. Hydrogel containing the charged biopolymers usually did not experience phase separation. They remained as a monolith, being unchanged with time. To achieve the same effect in the absence of polysaccharides, it is necessary, as shown in ref 13, to mix THEOS with a small amount of charged precursor, *N*-[(trimethoxysilyl)propyl]-*N,N,N*-trimethylammonium chloride. It seems that this role in the hybrid biomaterials is taken by the biopolymer.

An exception to the charged polysaccharides was represented by kappa-carrageenan. Its initially synthesized monolithic hydrogel was found with separated water in the days following preparation.¹⁴ The shrinkage continued within 5–7 days. The observed syneresis resembles that going on in the gels of kappa-carrageenan alone (see, for instance, ref 19). This fact means that polysaccharides determine the properties of hybrid materials in a significant manner.

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The optical transparency of hydrogels (last column of Table 1) was also dependent on the polysaccharide type. The uncharged polysaccharides made them turbid or opaque. The opalescence was usually observed for hydrogels containing anionic polysaccharides. It was possible to prepare a transparent material when cationic biopolymers were applied. The differences in the appearance of the hybrid hydrogels containing various polysaccharides may be caused by varying electrostatic interactions between the macromolecules and the silicic acid generated in situ. In addition, inorganic ions introduced as counterions with polysaccharides could have a notable effect on silica nucleation and growth. Their notable influence was pointed out in ref 1.

A distinguishing feature of the polysaccharides is that they sharply accelerated the kinetics of sol–gel processes. A hydrogel could settle within a few minutes after their addition, whereas it took at least few hours when such acceleration is caused by a catalytic effect of polysaccharides on the silica synthesis.

The sol–gel processes proceed through an initial hydrolysis of the silica precursor in a water solution and following condensation of the formed monomers (silanols). As a result, oligomers are produced. They are arranged as sol particles of nanoscale dimension. The processes come to a halt at this stage as long as a catalyst has not been introduced.^{10,11,20} To facilitate the cross-linking of sol particles leading to the sol–gel transition and formation of nanocomposite, an acid, alkali, phosphate, or fluoride salt is commonly introduced into the solution to provide a catalytic effect (see, e.g., refs 10 and 20). We found that mixing of THEOS with an aqueous solution of polysaccharides was sufficient to promote the synthesis of biomaterials. The sol–gel processes proceeded without addition of the above-mentioned catalysts. Moreover, the hydrogels settled through shorter time. It is also worth mentioning that it took place at ambient temperature and neutral pH of aqueous solutions at which the sol–gel transition is not observed in the case of currently used precursors.^{10,11}

Insight into the plausible role of polysaccharides in the sol–gel processes was provided when hybrid materials were examined with the help of a scanning electron microscope (SEM). Figure 1A demonstrates a SEM picture of an aerogel sample synthesized from THEOS alone. To promote the sol–gel processes, sulfuric acid was added as the catalyst. As seen in the picture, a three-dimensional network consists of solid particles connected to each other. Their diameter is varied between 10 and 40 nm. This type of network structure is typical of silica nanocomposite materials synthesized by sol–gel processing, which is in line with observations of other authors (see, e.g., ref 13).

Figure 1B gives an example of the structural organization of gel that was prepared by introducing THEOS into an aqueous solution of kappa-carrageenan. The synthesis was performed at pH ca. 6 without addition of acid to catalyze the processes. Easily one can see a large difference from the previous picture. There is also a three-dimensional network in Figure 1B, but it is made up of crossed or branched fibers. This features a fibrous network structure observed in kappa-carrageenan solutions, as shown, for example, in refs 21 and 22 with the help of electron and atomic force microscopy.

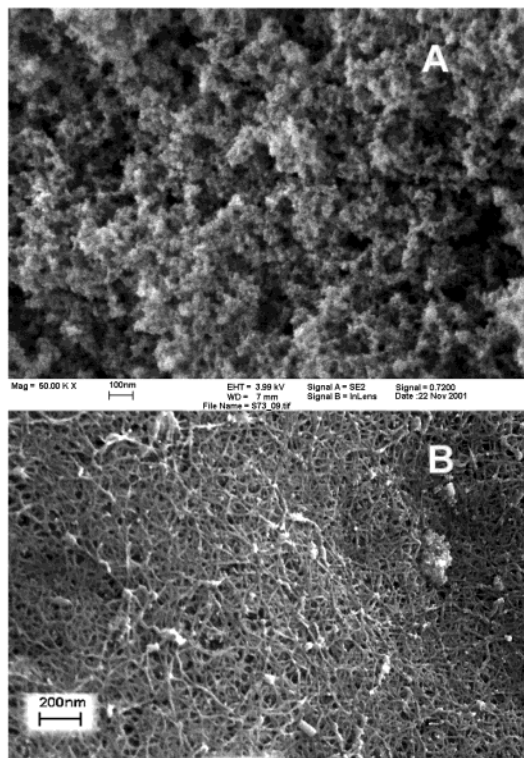


Figure 1. SEM micrographs of aerogel synthesized in an aqueous solution containing (A) 50 wt % THEOS, 0.2 wt % *N*-[(trimethoxysilyl)propyl]-*N,N,N*-trimethylammonium chloride and 0.1 M sulfuric acid and (B) 10 wt % THEOS and 0.5 wt % of kappa-carrageenan.

Figure 2 shows a set of SEM pictures for two hydrogels containing 1.8 wt % of cat-HEC (A,B) or xanthan (C,D). In contrast to the sample shown in Figure 1B, water was not exchanged for acetone at the sample preparation stage for microscope observation. In both cases a cryotechnique was applied. As seen (Figure 2A and C), the main morphological element of hydrogels is a cross-linked network. It is formed not by naked polysaccharide macromolecules, but by ones covered by silica. This is clearly recognized in the SEM pictures taken with higher magnification (Figure 2B and D). In addition, another morphological element present in the hydrogels is worth mentioning. There are spherical particles that fill the mesh space. They are also made up of silicate. It is reasonable to point out that the connections between the particles are not evident in most cases. It seems that cross-links between them are absent. This explains why the particles were not found in the sample shown in Figure 1B. This absence could be caused by their washing out of the sample during the water exchange for acetone.

Figure 3 demonstrates SEM pictures of biomaterials in which the concentration of polysaccharide (cat-HEC) is decreased from 1.8 (A) to 0.5 (B) wt %. The amount of precursor added in the initial solution was 10 wt % in both the cases. The main morphological elements, as previously, are a network and spherical particles. When the pictures are compared, it is apparent that the network became looser and the filaments thicker with decreasing polysaccharide concentration. It seems that only the spherical particles were not notably changed.

The influence of macromolecule charge density on the structure of biomaterial is obvious from Figure 4. There

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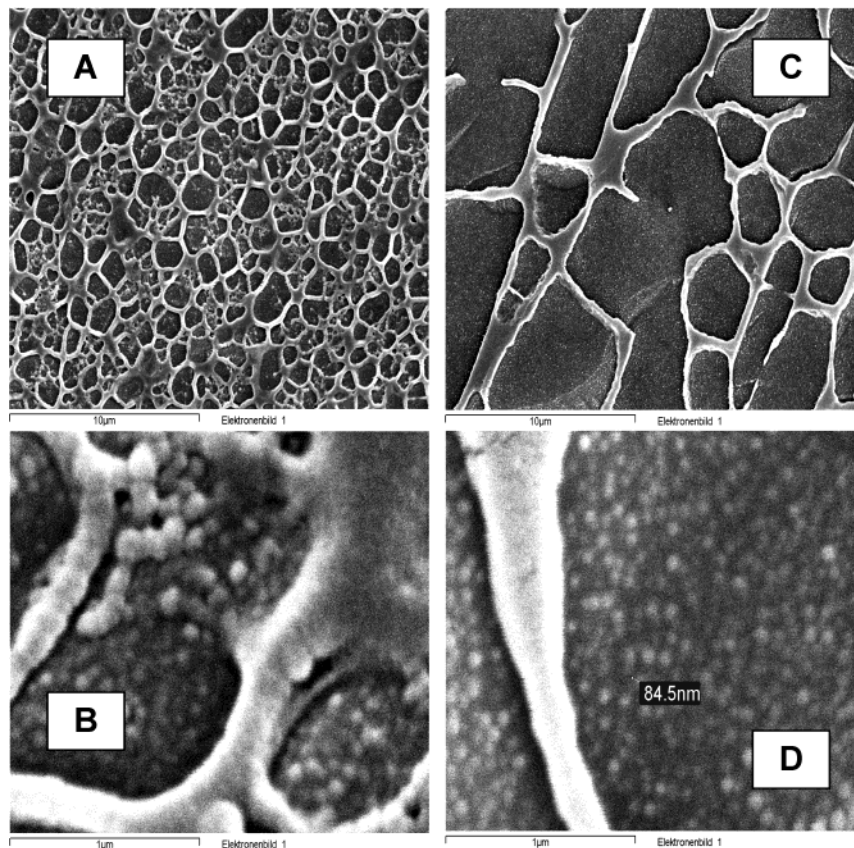


Figure 2. SEM micrographs of hydrogel synthesized in an aqueous solution of (A, B) cat-HEC (0.2 charged groups per monomer residue) and (C, D) xanthan. The concentration of polysaccharides was 1.8 wt % and THEOS 10 wt %.

are SEM pictures of two samples. They were synthesized by using the same concentrations of THEOS (10 wt %) and polysaccharide (1 wt %) as that of cat-HEC with different charge density in a macromolecule (Table 1). With exchanging a low-charged biopolymer (A) with the higher-charged one (B), one can find a biomaterial with a looser network. Careful examination of the SEM pictures shows that the morphological changes resemble those observed previously in the example of the concentration decrease (Figure 3), but the variation of charged degree induces larger modification of the network than the concentration. This points to the significant role of electrostatic interactions in the formation of hybrid material.

The above-considered results suggest that polysaccharide macromolecules go to work as a template for silicate generated by THEOS in the biopolymer solution. This means that their catalytic effect might be related to the interactions with silanols ($\equiv\text{Si}-\text{OH}$) produced in the course of hydrolysis of the precursor. The effect of charged groups was mentioned in the previous paragraph. However, the electrostatic interactions are not primarily responsible for acceleration of the sol–gel processes. It seems that they mainly influence the network structure and particle dimension in the biomaterials. The suggestion follows from the dependence of network density on the charged degree of macromolecules (Figure 4) and various optical properties of synthesized hydrogel (Table 1). If the electrostatic interactions could determine the catalytic effect on the sol–gel processes, one would expect a difference in the jellification time between systems containing oppositely charged polysaccharides. This was not found. Therefore, the reason for the acceleration of the sol–gel processes is in other interactions.

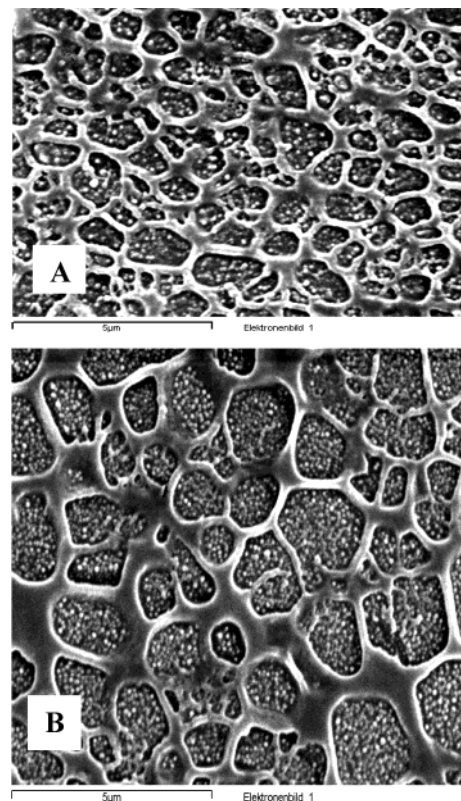


Figure 3. SEM micrographs of hydrogel synthesized in an aqueous solution of cat-HEC containing 0.06 charged groups per monomer residue. The concentration of THEOS was 10 wt % and polysaccharide 1.8 (A) or 0.5 (B) wt %.

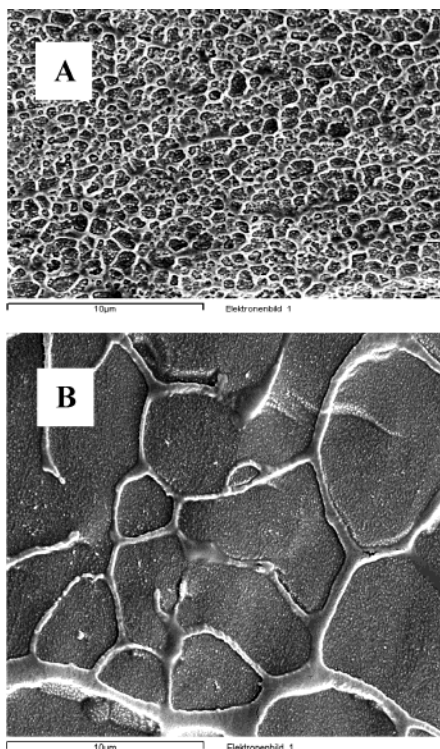


Figure 4. SEM micrographs of hydrogel synthesized in an aqueous solution of cat-HEC containing 0.06 (A) or 0.2 (B) charged groups per monomer residue. The concentrations of THEOS and polysaccharides were, respectively, 10 and 1 wt %.

We noticed that the polysaccharides are polyhydroxy compounds because they are composed of numerous monosaccharide residues (Table 1). Their hydroxyl groups could form hydrogen bonds or enter into the condensation reaction with silanols produced in the course of hydrolysis of the precursor, thus providing silica nucleation on macromolecules.

To test this assumption, THEOS was added into aqueous solutions of PEO and PVA. The synthetic polymers differ by the number of hydroxyl groups in their macromolecules. The high-molecular weight PEO contains only the terminal HO groups. Their number is negligible in comparison with that in PVA in which macromolecules the hydroxyl group is almost attached to each monomer residue.

The testing showed that a hydrogel was formed only in the solution containing PVA. The sol–gel transition was not observed where THEOS was mixed with PEO. In this case, a precipitate was formed, but the viscosity of the solution was not notably changed.

The essential difference found between PEO and PVA in their effect on THEOS behavior in aqueous solutions makes it obvious that catalysis of the sol–gel processes by naturally occurring polysaccharides is caused by numerous hydroxyl groups in their macromolecules. In our opinion, silanol ($\equiv\text{Si}-\text{OH}$) produced after the hydrolysis of the precursor might form hydrogen bonds with the corresponding groups of biopolymers. Another opportunity consists of covalent bonding with the HO groups. However, polysaccharide macromolecules, as established for carrageenans in our previous article,¹⁴ retained their ability to conformational transitions in the hybrid biomaterials. This is possible if they do not interact strongly with silanols. The covalent bonds are formed by silanol molecules, which provides the nucleation and generation of the silica shell around the macromolecules.

It is worth mentioning that the idea of hydrogen-bond formation between biopolymers and silicate generated by biomineralization processes in the living cells has been discussed in the literature by various authors (see, e.g., reviews in refs 1–3). It is believed that hydrogen-bonding units of macromolecules or their assemblies might serve as nucleating centers for silicate, thus regulating its synthesis. The above-considered results give experimental evidence for the validity of this idea.

The foregoing results showed (Figures 1–4) that the structure of synthesized biomaterials is manipulated by the polysaccharides. The variations in their type, concentration, or charge density in the macromolecule result in a change of the density of the network. It is reasonable to expect that their mechanical properties are also regulated by the polysaccharides.

The corresponding experimental results are given by an example of alginate in Figure 5A. Shown are the zero-shear viscosity η_0 (curve 1) and yield stress σ_y of hydrogel (curve 2) versus the polysaccharide concentration. The THEOS amount in the initial solution was equal to 10 wt %. The alginate effect on the rheological properties become obvious at concentrations exceeding 0.5 wt %. As seen, both the zero-shear viscosity and yield stress are decreased with increasing the polysaccharide amount in the hydrogel. In other words, the alginate made it softer and less rigid and hard. An analogous effect on the mechanical properties of hydrogels was found where all the polysaccharides listed in Table 1 were examined.

The silicate tends to an opposite effect. This is obvious from Figure 5B. An increase of precursor concentration in the initial solution brings about a sharp rise in the zero-shear viscosity (curve 1) and yield stress (curve 2) of hydrogel. The change of rheological parameters is proportional to the added THEOS amount. The power law exponents for concentration dependencies, which were found by the best least-squares fits to the experimental

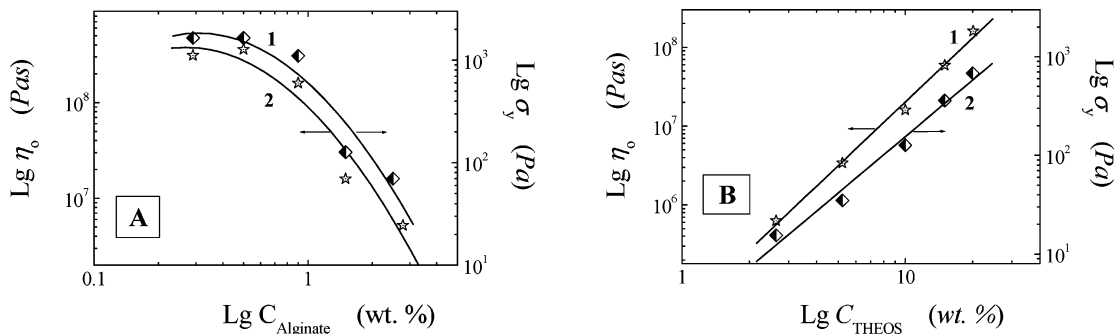


Figure 5. Zero-shear viscosity (1) and yield stress (2) vs the concentration of alginate (A) or THEOS (B) in the initial aqueous solution. The concentration of THEOS was 10 wt % (A) and alginate 1.5 wt % (B).

Table 2. Values of the Exponents

polysaccharide	concentration, wt %	zero-shear viscosity	yield stress
alginate	1.5	2.7 ± 0.1	1.9 ± 0.2
iota-carrageenan	1.5	3.2 ± 0.2	2.1 ± 0.3
cationic derivative of hydroxyethylcellulose, cat-HEC	0.2	3.9 ± 0.8	2.2 ± 0.4

data, are equal, respectively, to 2.7 and 1.9. This is an indication of a strong sensitivity of mechanical properties to the silica amount produced in the polysaccharide solution. It makes the hydrogel more hard and rigid. The reason for the reinforcing effect of silicate is attributable to the formation of a shell surrounding the macromolecules (Figures 1–4). It strengthens them mechanically.

The reinforcing effect of silicate on the hydrogels was found for all polysaccharides examined (Table 1). However, there were variations in the concentration dependencies of rheological parameters. Values of the power law exponents determined from the corresponding experimental data are presented in Table 2. One may see how they vary somewhat from sample to sample. It seems that the exponent values also depend on the polysaccharide concentration in the biomaterial. This follows from data obtained with various amounts of cationic derivative of HEC. As seen, a decrease of the polysaccharide amount results in an increase in the exponents. The reason for the effect of polysaccharide type and concentration on the slope of concentration dependences has to do with the differences

in the network structure, but we do not have enough data to correlate them.

Conclusions

The foregoing results allow the following conclusions to be made. (1) The completely water-soluble precursor made it possible to synthesize new monolithic nanocomposite silica biomaterials on the basis of various polysaccharides by means of sol–gel processing in aqueous solutions without the addition of an organic solvent and a catalyst. (2) The polysaccharides promoted silica polymerization through acceleration and catalysis of processes. (3) Their effect is explicable by the formation of hydrogen bonds between hydroxyl groups of macromolecules and silanols generated by the hydrolysis of precursor. (4) The polysaccharides served as a template for silica generated in situ. (5) The structure and properties of synthesized biomaterials depended on the polysaccharide type, charged degree of their macromolecule, and concentration, which provides a means of manipulating the structural organization of hybrid polysaccharide–silica nanocomposites.

Acknowledgment. The authors are indebted to Hercules Copenhagen A/S (Denmark) for a sample of lambda-carrageenan. The help of Mr. M. Meyer (Bayreuth University) in the preparation of aerogels and of Dr. C. Abetz and Ms. I. Otto (Bayreuth University) in the SEM micrographs of biomaterials is gratefully acknowledged.

LA0356912