

# Sol–gel-derived biomaterials of silica and carrageenans

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## Abstract

A new precursor, tetrakis(2-hydroxyethyl) orthosilicate (THEOS), introduced by Hoffmann et al. (J. Phys. Chem. B 106 (2002) 1528–1533), was used to synthesize monolithic hybrid biomaterials on the basis of silica and three main types of carrageenans,  $\kappa$ -,  $\iota$ -, and  $\lambda$ -carrageenans. The advantage of THEOS over the currently applied TEOS and TMOS is in its complete solubility in water. This negated the need to add organic solvents, thus excluding a denaturing effect on biopolymers. In their turn, carrageenans introduced into the precursor solution made use of common catalysts unneeded to trigger the sol–gel transition. It was found that they promoted the mineralization, acting as a template for the inorganic component. The kinetics of sol–gel processes, mechanical properties, phase behavior, and structure of novel hybrid biomaterials were studied by dynamic rheology, differential scanning calorimetry, and scanning electron microscopy. The material properties were regulated by both the precursor and carrageenan. The increase of silicate concentration led to a rise in the stiffness and brittleness of the material, whereas the polysaccharide addition made it softer and more elastic. It was shown that the formation and properties of mixed gels were determined by the nature of carrageenan.  $\kappa$ -Carrageenans brought about shrinkage of hybrid materials that led to water separation, while  $\iota$ - and  $\lambda$ -carrageenans did not induce the syneresis. This is in line with the difference in polysaccharide properties when they are in aqueous solutions without silicate. Furthermore,  $\kappa$ - and  $\iota$ -carrageenans experienced a thermoreversible phase transition in the hybrid materials owing to the helix–coil transition. This resulted in a step like change in the mechanical properties of mixed systems in the corresponding temperature range.  $\lambda$ -Carrageenan is a nongelling polysaccharide, which is why the rheological parameters of its hybrid gel were unchanged with the temperature. It was found that the polysaccharides modified the structure of silica-based materials. They transformed a three-dimensional network of connected silica particles into that consisting of crossed fibers.

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## 1. Introduction

The biopolymer-containing hybrid nanocomposite materials of silica prepared with the help of sol–gel processes in aqueous solutions have drawn attention owing to their promising properties and biocompatibility with living matter [1–9]. Tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS) are currently used as precursors. It is believed that the inorganic nanocomposite is formed in situ in a biopolymer solution by the self-organization of sol particles generated in the course of hydrolysis of TMOS or TEOS and following polycondensation reactions into a porous three-dimensional network in the bulk solution. The disadvantage of these precursors is that they are poor-water-soluble com-

pounds. To increase the solubility, methanol or ethanol is usually added [10]. In addition, an alcohol is issued as a result of tetraalkoxysilane hydrolysis. Both TMOS or TEOS and alcohols demonstrate poor biocompatibility and denaturing effect on biopolymers as well as decrease their solubility [3,11]. This imposes some constraints on the preparation of hybrid materials.

The biocompatibility of silica precursor was improved, as shown by Gill and Ballesteros [11], when polyol silicates were examined. It was also shown recently in [12] that tetraalkoxysilanes can be successfully exchanged for tetrakis(2-hydroxyethyl) orthosilicate (THEOS). The advantages were established for this precursor. THEOS was completely water-soluble. It allowed synthesis of monolithic porous materials in which aging was not accompanied by syneresis when only tenths percent of *N*-[(trimethoxysilyl)propyl]-*N,N,N*-trimethylammonium chloride was introduced into the reaction mixture.

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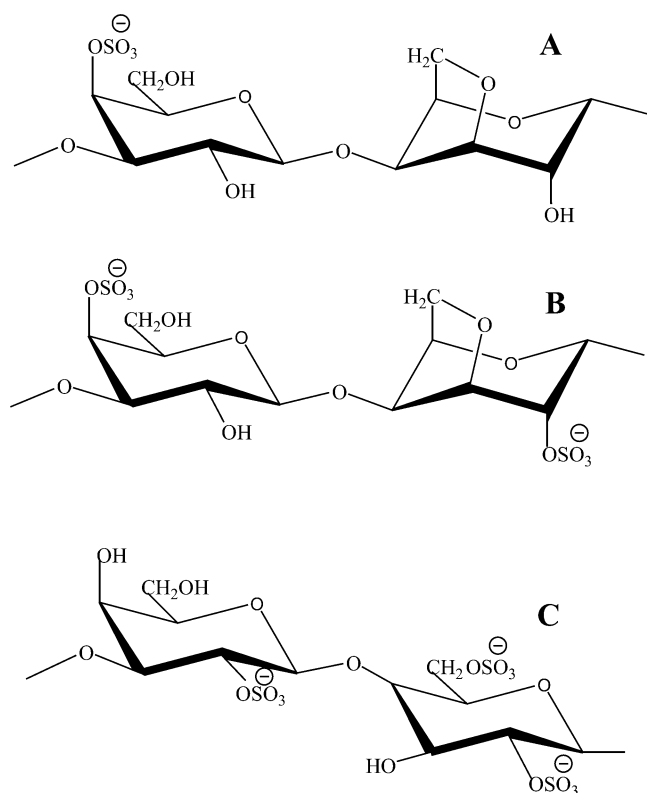


Fig. 1. Structural formula of  $\kappa$ - (A),  $\iota$ - (B), and  $\lambda$ - (C) carrageenans.

The main objectives of the present article were to examine the THEOS as a precursor for preparing hybrid silica biomaterials with carrageenans and study how the nanocomposite formation, properties, and structure depend on the introduced polysaccharides and their type.

In deciding on carrageenans, it was taken into account that this group of polysaccharides includes species possessing various gel-forming abilities. The most common types of carrageenans are  $\kappa$ -,  $\iota$ -, and  $\lambda$ -ones [13–16]. They consist of regularly alternating (1  $\rightarrow$  3)-linked  $\beta$ -D-galactopyranose and (1  $\rightarrow$  4)-linked  $\alpha$ -D-galactopyranose residues, which is why they are called galactans. The  $\alpha$ -D-galactopyranose residue is predominantly in 3,6-anhydro form in  $\kappa$ - and  $\iota$ -carrageenans. Their repeating disaccharide units are presented in Fig. 1. The 4-positions of  $\beta$ -D-galactopyranose residue is almost fully sulfated in these polysaccharides.  $\iota$ -Carrageenan contains additionally a sulfo group in the 2-position of the anhydrogalactose residue or it can be substituted for  $\alpha$ -D-galactose-6-sulfate or  $-2, 6$ -disulfate. Both these polysaccharides are capable of forming a thermoreversible gel in aqueous solutions. This is related to a coil-helix transition of their molecules [13–16].  $\gamma$ -Carrageenan is a nongelling galactan. Its molecules, distinct from respective counterparts ( $\kappa$ - and  $\iota$ -ones), does not contain 3,6-anhydro- $\alpha$ -D-galactopyranose residues.  $\gamma$ -Carrageenan is the most sulfated type among this group of polysaccharides. Sulfate groups are usually found in the 4 position of a  $\beta$ -D-galactopyranose residue and in the 2- and 6-positions of a  $\alpha$ -D-galactopyranose residue. The rheological properties of

aqueous solutions of carrageenans are quite distinct [13–16]. The hardest, strongest, and most brittle gels are produced by  $\kappa$ -carrageenan.  $\iota$ -Carrageenan gives soft and weak gelled solutions, whereas  $\gamma$ -carrageenan is capable only of inducing a thickening effect in its solutions.

An opportunity for the formation of hybrid silica materials with a number of polysaccharides has been noted in [3,11]. Mention was also made of  $\kappa$ -carrageenan, but information on the properties of these nanocomposites was not given.

Detailed results on biomaterials containing the main types of carrageenans are presented in this article. It was shown that the THEOS and biopolymers are compatible in aqueous solutions, mixing without phase separation. This enabled me to synthesize a set of hybrid nanocomposites. The silica generated in situ reinforced gel structures of polysaccharides. In their turn they serve as a template for the generated silica, providing a change in the gel morphology. Furthermore,  $\kappa$ - and  $\iota$ -carrageenans induce thermal reversibility in the mechanical properties of the gelled hybrid materials, which can be related to the polysaccharide helix-to-coil transition. A possible mechanism for the bio-nanocomposite formation is suggested.

## 2. Experimental

### 2.1. Materials and methods

The precursor tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was prepared by using tetraethoxysilane (ABCRC, Germany) as described in detail in [17].  $N$ -[(Trimethoxysilyl)propyl]- $N, N, N$ -trimethylammonium chloride was obtained from ABCRC.  $\kappa$ - and  $\iota$ -carrageenans were purchased from Fluka. Their molecular weight, as determined by size exclusion chromatography with a Shimadzu LC-6A chromatograph by Dr. S.V. Sukhoverkhov, was equal to about 700 kDa.  $\kappa$ -Carrageenan contained low-molecular-weight impurities (97 kDa and 340 Da).  $\gamma$ -Carrageenan (1024 kDa, high degree of purification) was a gift of Hercules Copenhagen A/S (Denmark). The polysaccharides were used as supplied. Acetone was from Fluka. Water was purified by double distillation.

Nanocomposite materials were prepared in accordance with the procedure suggested in [12], except that carrageenans were introduced into the reaction mixture. In the beginning the polysaccharides were dissolved in water and left for a day. The weighed appropriate amount of precursor was added into their aqueous solutions. To prepare the unmodified silica nanocomposite, sulfuric acid,  $N$ -[(trimethoxysilyl)propyl]- $N, N, N$ -trimethylammonium chloride, and THEOS were mixed with water. In either case, the mixtures were thoroughly stirred for ca. 5 min and allowed to stay at ambient temperature at least for a week prior to examination of its properties. Some samples were re-examined within 9 months.

Rheological measurements were performed with a Bohlin CS-10 or Rotovisco RT 20 (Haake) stress-controlled rheometer. Measuring cells had cone-and-plate geometry. The rheometers were run in either an oscillation or creep regime. The oscillatory frequency was varied from 0.001 to 10 Hz. The creep regime was used to determine a zero-shear viscosity and plateau modulus. These measurements were performed at  $25 \pm 0.05$  °C. Temperature dependencies of rheological parameters were measured at 1 Hz within a certain time interval, while the temperature is varied at 2 °C a minute.

The calorimetric measurements on the samples examined in parallel by rheology were carried out with the help of a micro differential scanning calorimeter micro DSC III from Setaram (France). Then 0.8 to 0.9 g of a gel was sealed hermetically into a pan, while a pan containing approximately the same amount ( $\pm 20$  mg) of doubly distilled water was used for the reference. Both pans were placed into the calorimeter and cooled up to 1 °C. To annihilate the thermal history and equilibrate the samples, they were kept for 60 min. Then the temperature was raised up to 80 °C at  $0.1$  °C min<sup>-1</sup> and after 15 min of exposure it was lowered up to 1 °C with the same rate. The heating–cooling procedure recurred once again within 30 min.

To take SEM micrographs, aerogels were prepared by means of a two-step procedure. At first the water was substituted for acetone by placing fivefold excess of the organic solvent into the bottle with a gel and then exchanging it for its fresh portion five times. It took about a month. At the final stage acetone was removed by hypercritical drying. The surface of dried samples was covered by platinum layer of ca. 1  $\mu$ m in thickness. The pictures were made with a FE-SEM Leo 1530 electron microscope.

## 2.2. Data analysis

### 2.2.1. Creep and recovery

This regime was used to find the zero-shear viscosity  $\eta_0$  and the plateau modulus  $G_0$  to characterize quantitatively the mechanical properties of synthesized gels. Figure 2 gives an example of a sample subjected to a constant shearing stress,  $\sigma_0$ , that results in the development of strain,  $\gamma$ , in it with time. This is a creep region. It is followed by a recovery region that is observed after the cessation of the stress. Additional notes and constructions explaining how the zero-shear viscosity and plateau modulus were determined. The creep regime can be described by the equation [18–20]

$$\gamma(t) = \frac{\sigma_0}{G_0} + \frac{\sigma_0}{\eta_0}t, \quad (1)$$

where the first term is associated with an elastic response of material and the second term with the viscous flow. In the recovery region, one may see only the elastic response.

### 2.2.2. Stress sweep

This regime was used to find the critical stress  $\sigma_y$  (yield stress value) and strain  $\gamma_{cr}$ , at which the gel flowing or break-

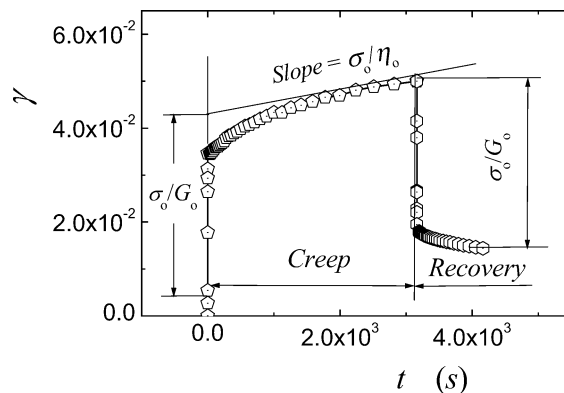


Fig. 2. Creep and recovery after application and cessation of a step stress  $\sigma_0$  of 4700 Pa. Explaining notes and constructions show how the rheological parameters are determined. The measurements were performed with a gelled solution containing 50 wt% THEOS, 0.2 wt% *N*-[(trimethoxysilyl)propyl]-*N*, *N*, *N*-trimethylammonium chloride, and 1 M sulfuric acid.

ing occurs under the action of an external mechanical force. The shear stress was applied in an oscillation regime at the frequency of 1 Hz by the progressive increase of its value, while the strain and dynamic moduli were measured. A  $\sigma$ – $\gamma$  dependence is presented in Fig. 3A. The initial portion of the curve, where the proportionality between stress and strain is, refers to linear viscoelastic behavior. The dynamic moduli in this region are independent of the strain. A corresponding dependence for storage  $G'$  and loss  $G''$  moduli is shown in Fig. 3B. As the  $\sigma$  is over the linear limit, the moduli both become strain-dependent. This is a region of nonlinear response. It should be pointed out that at the beginning stage of this region the storage modulus is still higher in magnitude than the loss one. With increasing the stress, the curves meet each other, whereupon the  $G''$  stands out above the  $G'$ . The intersection point testifies for a change in the rheological behavior of the sample, determining the critical strain and stress values at which flow begins. The corresponding  $\sigma_y$  is usually called the yield stress in the literature (see, e.g., [21]). Such plots as presented in Fig. 3 were used to find first  $\gamma_{cr}$  and then  $\sigma_y$ , thus characterizing the mechanical destruction of synthesized gels under the external mechanical force.

## 3. Experimental results

### 3.1. Kinetics and conditions for the sol–gel processing

An initial solution of the precursor, as soon as THEOS has been mixed with acidified water, is a low-viscous liquid of which the viscosity normally ranges from  $10^{-3}$  to  $10^{-2}$  Pa s. Figure 4 shows changes in the storage modulus  $G'$  and loss modulus  $G''$  with passing time. This includes at least three basic stages. At the first one the dynamic moduli are almost unchanged in time. After this initial lag period there is a sudden sharp increase in the rheological parameters that represent a gel development stage. The time at which the

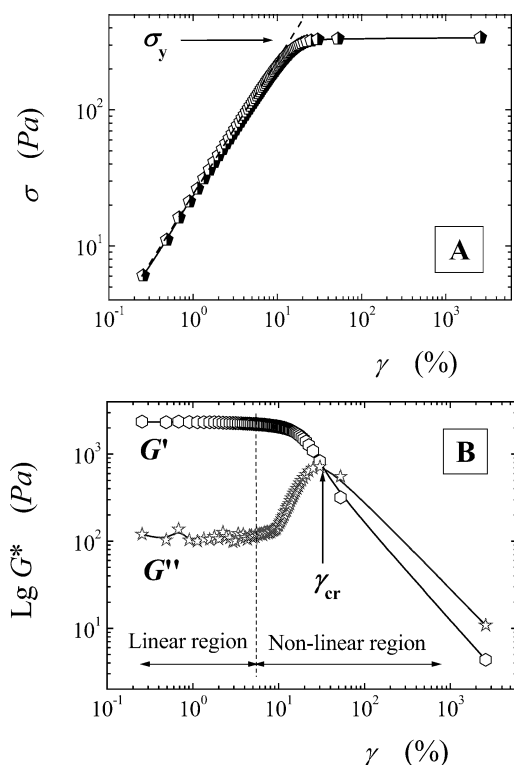


Fig. 3. (A) The stress applied at the frequency of 1 Hz vs strain  $\gamma$ . An arrow marks the critical stress (yield) value. (B) The storage modulus  $G'$  and loss modulus  $G''$  vs strain  $\gamma$  measured at oscillation frequency of 1 Hz. An arrow marks a critical strain, at which a transition takes place to a new rheological regime. The gel was prepared in an aqueous solution containing 10 wt% THEOS, 0.15 wt%  $N$ -[(trimethoxysilyl)propyl]- $N,N,N$ -trimethylammonium chloride, and 1 M sulfuric acid.

transition into a gel state happens follows from a crossover of storage and loss moduli curves [22]. Then the  $G'$  becomes larger than  $G''$  beyond the crossing. This signifies a transition to the state with a new rheological regime with features obvious from frequency dependencies of the rheological parameters presented in Fig. 5. The complex viscosity  $|\eta^*|$  varies in inverse proportion to the oscillation frequency  $f$ , whereas  $G'$  is higher in magnitude than  $G''$  and they both are almost independent of  $f$ . These properties are typical of soft or semisolid materials [23] to which the silica gels prepared by the sol–gel procedure can be assigned.

After the initial sharp growth of the rheological parameters at the second stage (Fig. 4) one can observe a gradual decrease in the rate of their increase and then the curves level off with the passage of time. This is indicative of a changeover to the mature or cured gel. This represents a final, third, stage in the gelling process.

The carrageenans influenced notably the kinetics of sol–gel processes. They all induced a sharp decrease in the duration of the first stage (Fig. 4). The viscosity of the solutions began rising within a few minutes after the addition of carrageenans, which depended on their and THEOS concentrations.

The polysaccharides affected also the conditions for the gel preparation. The acceleration of the kinetics of sol–

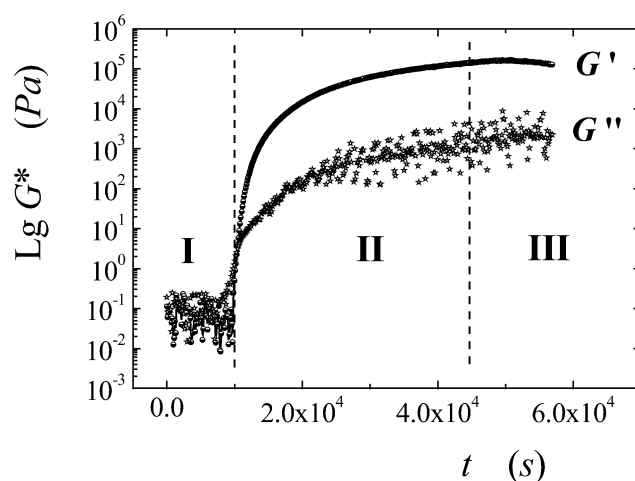


Fig. 4. Time dependencies of the shear moduli measured at a frequency of 1 Hz. An initial aqueous solution consists of 50 wt% of precursor, 0.2 wt%  $N$ -[(trimethoxysilyl)propyl]- $N,N,N$ -trimethylammonium chloride, and 1 M sulfuric acid. Roman numbers marks the stages.

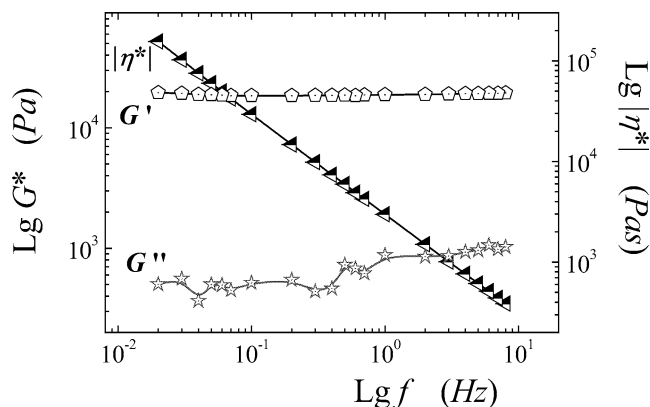


Fig. 5. The complex viscosity  $|\eta^*|$ , storage modulus  $G'$ , and loss modulus  $G''$  vs oscillation frequency  $f$ . An initial aqueous solution contained 50 wt% THEOS, 0.2 wt%  $N$ -[(trimethoxysilyl)propyl]- $N,N,N$ -trimethylammonium chloride, and 1 M sulfuric acid.

gel processes was caused by their catalytic effect. This allowed nonusage of common catalysts, acid in particular, for providing the gellification of solutions. Furthermore, carrageenans influenced the syneresis of gelled mixtures. This depended on the polysaccharide type. Samples containing  $\iota$ - or  $\gamma$ -carrageenan left as the monolith during all the examined time intervals. The separated solvent was not found within 9 months. This was observed even in the absence of  $N$ -[(trimethoxysilyl)propyl]- $N,N,N$ -trimethylammonium chloride. The addition of  $\iota$ - and  $\gamma$ -carrageenans makes its use unnecessary for preparation of a monolithic gel.

The syneresis took place when only  $\kappa$ -carrageenan was included in the hybrid materials. The initially synthesized monolithic nanocomposite experienced shrinkage in the next day after the preparation. The maturation of gel was accompanied by further solvent separation. This proceeded for about a week. Such behavior of hybrid nanocompos-

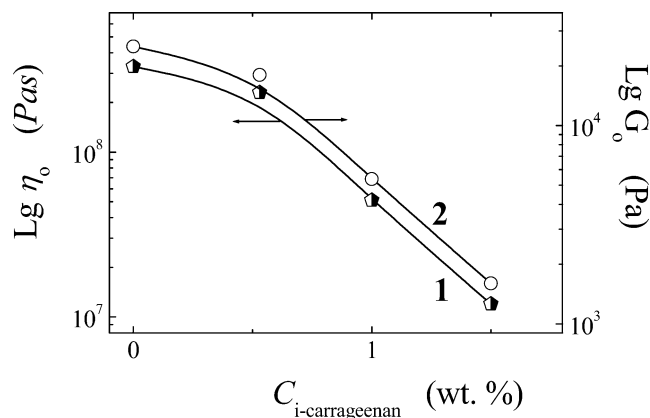


Fig. 6. The zero-shear viscosity (1) and plateau modulus (2) vs the concentration of  $\iota$ -carrageenan. The concentration of THEOS was 10 wt%. The biomaterial was prepared without the addition of sulfuric acid and  $N$ -[(trimethoxysilyl)propyl]- $N,N,N$ -trimethylammonium chloride.

ite resembles that observed for the gels of  $\kappa$ -carrageenan alone [13–16].

### 3.2. Mechanical properties

Figure 6 presents the zero-shear viscosity and plateau modulus of gelled materials against the concentration of added  $\iota$ -carrageenan. Similar dependencies were found for the  $\kappa$ - and  $\gamma$ -carrageenan-containing nanocomposites. As obvious from Fig. 6, the polysaccharide started decreasing the zero-shear viscosity and plateau modulus of hybrid silica nanocomposites at the concentration of about 0.5 wt%. One can find more than a tenfold drop of these parameters at 1.5 wt% of  $\iota$ -carrageenan in the initial solution. Furthermore, the polysaccharide made the biomaterials less brittle. As seen in Fig. 7A, the critical strain  $\gamma_{cr}$  experienced a sharp increase. This means that the hybrid nanocomposite containing  $\iota$ -carrageenan became more elastic than the pure silicate nanocomposite. At the same time, the polysaccharide addition resulted in a decrease of the yield stress (Fig. 7B), i.e., the mechanical strength of the biomaterial.

Figure 8 shows how the zero-shear viscosity and plateau modulus of silica nanocomposites with 1.5 wt% of  $\iota$ -carrageenan depend on the precursor concentration in the initial solution. The monolithic gel started forming at about 4 wt% of THEOS. This is a soft, easily deformed material. An increase of THEOS concentration led to a sharp growth of the rheological parameters. The power law exponents for the concentration dependencies of  $\eta_0$  and  $G_0$  are equal to 3.2 and 4.0, respectively. The yield stress, as seen in Fig. 9, experienced also a similar sharp rise ( $\sigma_y \propto C^{2.7}$ ). All these facts are an indication of a strong dependence of the mechanical properties of biomaterials on the silica content in the gelled system. The inorganic component provides a strong reinforcing effect on solutions of all three carrageenans.

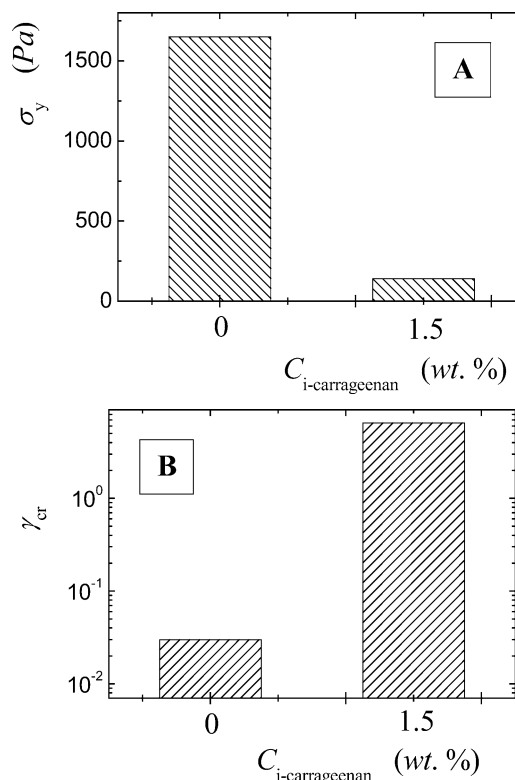


Fig. 7. The yield stress (A) and critical strain (B) vs the concentration of  $\iota$ -carrageenan. The concentration of THEOS was 10 wt%.

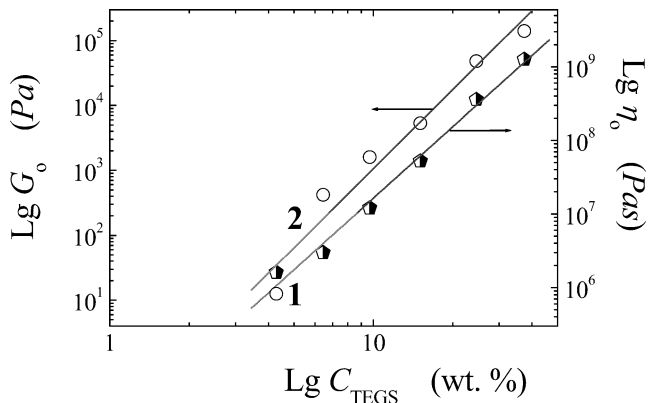


Fig. 8. The zero-shear viscosity (1) and plateau modulus (2) vs the concentration of THEOS in the initial solution. The concentration of  $\iota$ -carrageenan was 1.5 wt%.

### 3.3. Temperature effect

A steplike change of the complex viscosity with the temperature ( $T$ ) for a sample containing 1.5 wt% of  $\iota$ -carrageenan and 4.3 wt% of precursor is presented in Fig. 10A. This is observed in a narrow temperature range, while  $|\eta^*|$  is almost constant at  $T$  being outside of it. Therefore, it is reasonable to relate the change to a phase transition in the gelled system. Its temperature is easily determined when a dependence of  $\tan \delta$ , where  $\delta = G''/G'$ , versus the  $T$  is examined. It is plotted in Fig. 10B for a sample with an increased amount of THEOS (6.5 wt%) and the same

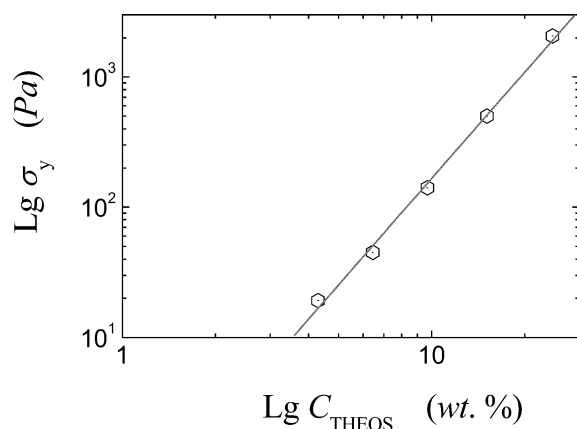


Fig. 9. The yield stress vs the concentration of precursor in the initial solution. The concentration of  $\iota$ -carrageenan was 1.5 wt%.

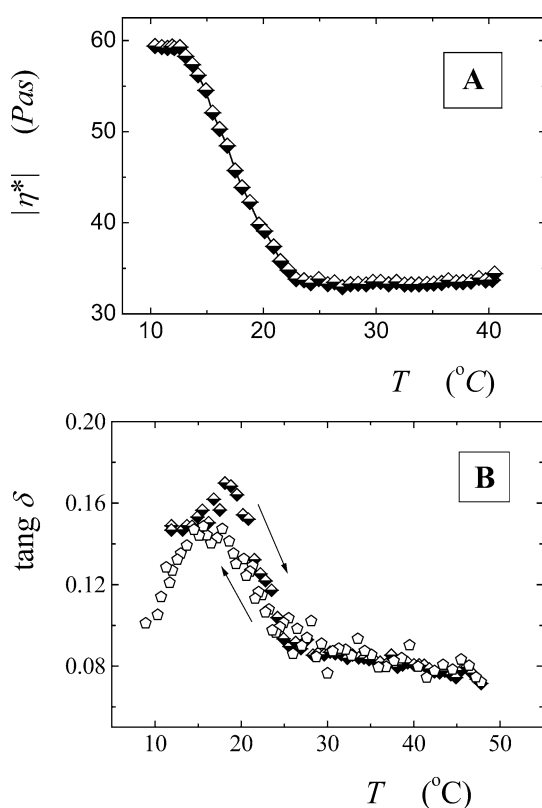


Fig. 10. The complex viscosity (A) and  $\tan \delta$  (B) vs the temperature for a gel obtained in the initial solution with 1.5 wt%  $\iota$ -carrageenan and 4.5 (A) or 6.5 (B) wt% THEOS. The  $|\eta^*|$  shows data for the sample being heated, while the shear moduli (B) were measured when the temperature, as shown by arrows, was increased or decreased.

concentration (1.5 wt%) of  $\iota$ -carrageenan. There are data obtained when samples were heated or cooled. One may see a peak in each of both the curves. This is indicative of the reversibility of transition, but there is a difference in the peak location, which is equal to a few degrees.

The reason for the phase transition can be caused by the presence of  $\iota$ -carrageenan. It is a well-known fact that this polysaccharide is capable of forming a thermoreversible gel

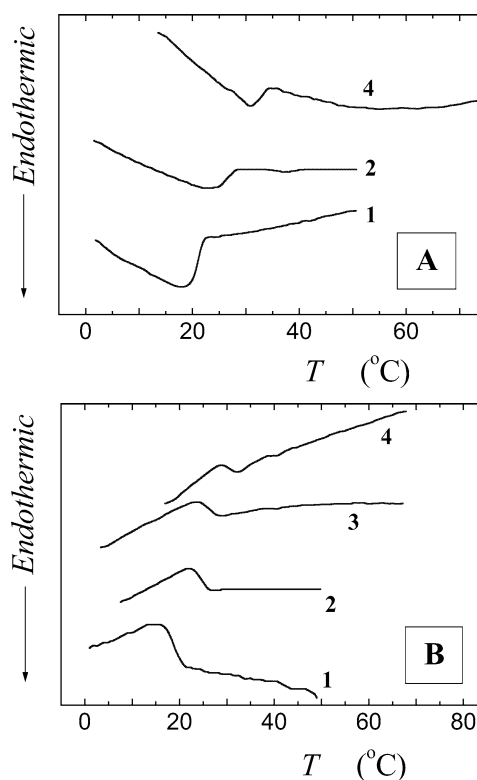


Fig. 11. Heating (A) and cooling (B) DSC curves of samples containing 1.5 wt% of  $\iota$ -carrageenan and 0 (1), 6.45 (2), 9.7 (3), and 24.6 (4) wt% of THEOS.

in the aqueous media [13–16]. The gellification is brought about by a coil–double helix transition. Figure 11 demonstrates heating (A) and cooling (B) DSC curves measured on samples containing 1.5 wt%  $\iota$ -carrageenan and various amounts of the precursor in the initial solution. For reference, curves 1 represent data on an aqueous solution containing  $\iota$ -carrageenan alone. As seen, the silica produced in situ resulted in a shift of both the endothermic and exothermic peaks in the curves into the high-temperature range. The more precursor added into the initial solution, the greater the effect. The melting and gel setting temperatures determined from DSC measurements are presented in Fig. 12A against the precursor concentration. There is a difference in 2–3 °C between the phase transition temperatures determined by the cooling and heating of samples. A similar difference was mentioned above in the case of rheological measurements (Fig. 10).

The next effect followed from the calorimetric data (Fig. 11) is that the peak is decreased with increasing precursor concentration in the initial mixture. This means that the heat of processes is decreased. The corresponding data presented as a concentration dependence of the enthalpy for the coil–helix transitions can be seen in Fig. 12B.

The above-considered temperature effects were observed for the hybrid nanocomposite materials containing  $\iota$ - or  $\kappa$ -carrageenan. They both possess an ability to form thermoreversible gels in aqueous solutions. When a biomaterial was prepared by introducing  $\gamma$ -carrageenan, the steplike changes

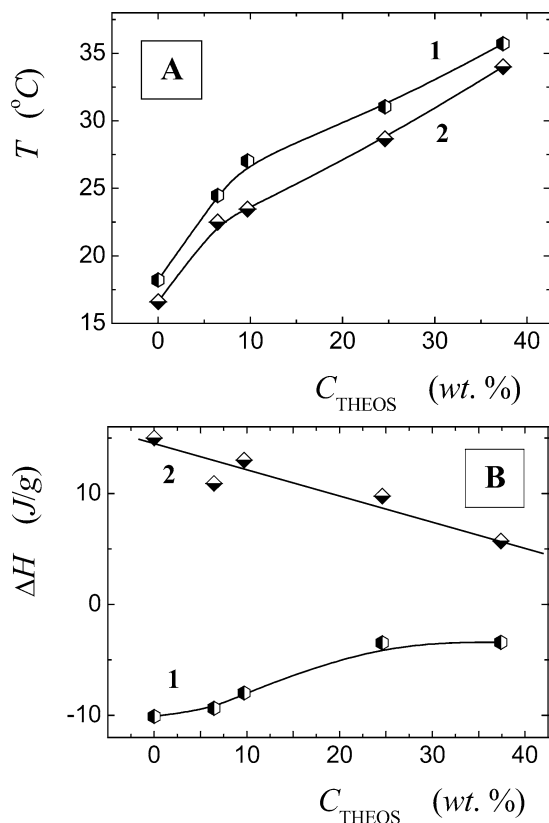


Fig. 12. (A) Endothermic (1) and exothermic (2) peak temperatures, respectively, in heating and cooling DSC curves (Fig. 11) and (B) the enthalpy of processes corresponding to them vs the precursor concentration. The concentration of  $\kappa$ -carrageenan was 1.5 wt%.

in the rheological parameters or the calorimetric effect were not found. This result is consistent with the absence of gelling properties for this polysaccharide [13–16].

### 3.4. Electron microscope observations

Figure 13 presents a FE-SEM picture of a gel sample prepared in the absence of polysaccharide in the initial solution. Solid particles can be seen. Their diameter is varied, as followed from tentative estimates, between 10 and 40 nm. The solid particles connect to each other, forming a three-dimensional network. This structure is typical of silica nanocomposite materials, being in line with observations of other authors (see, e.g., [12]).

A FE-SEM picture of a sample containing  $\kappa$ -carrageenan is given in Fig. 14. A great difference from the previous picture is obvious. There is also a three-dimensional network, but it is made up of crossed fibers. It should be stressed that the spherical particles are absent. The fibrous network structure found in the biomaterial resembles that existing in biopolymer solutions viewed with the help of electron or atomic force microscopy (see, for example, [24,25]). The absence of separated spherical particles, whereas the fibers are dominating morphological element, can mean that the silica formed in situ by the sol-gel processes is linked with

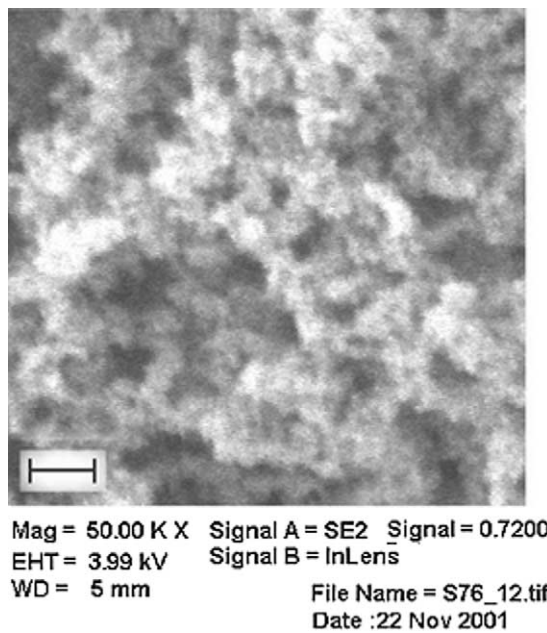


Fig. 13. A SEM micrograph of aerogel synthesized in an aqueous solution containing 50 wt% THEOS, 0.2 wt%  $N$ -[(trimethoxysilyl)propyl]- $N,N,N$ -trimethylammonium chloride, and 1 M sulfuric acid.

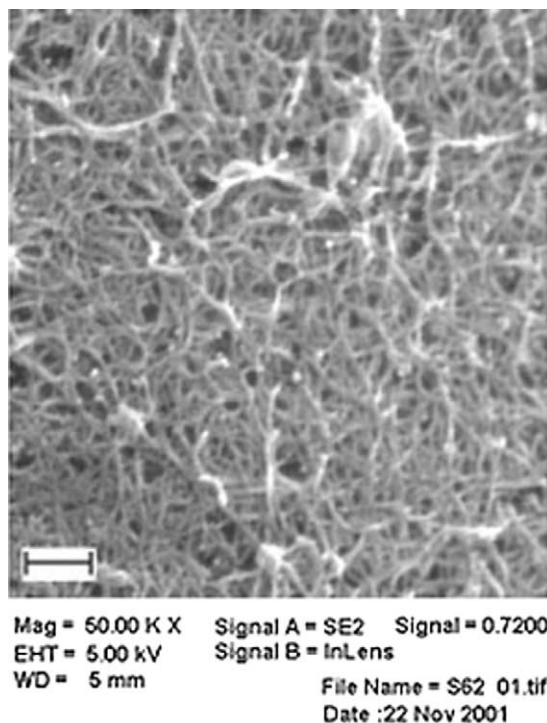


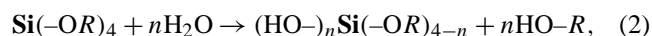
Fig. 14. A SEM micrograph of silica aerogel synthesized in the presence of 0.5 wt% of  $\kappa$ -carrageenan. The concentration of THEOS was 10 wt%.

polysaccharide macromolecules at the molecular scale. It is reasonable to suggest that the structural organization of biomaterials is dictated by carrageenans. Their chains serve as a template for synthesizing inorganic component.

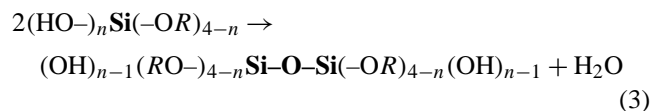
#### 4. Discussion

The foregoing results have shown that the mixing of silica precursor with carrageenans in the aqueous solutions results in the formation of homogeneous monolithic biomaterials. Their properties are regulated by both inorganic and organic components. This allows assignment of them to the hybrid materials.

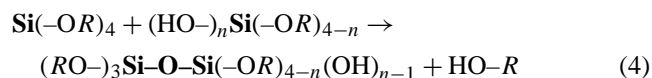
The commonly accepted mechanism for the formation of silica nanocomposite materials includes three main stages [10,26]: (1) hydrolysis of the precursor; (2) condensation of the formed monomers to produce oligomers arranged as sol particles; (3) cross-linking of the sol particles leading to a sol–gel transition. This stems from a set of chemical reactions, which are initiated by a catalyst in the precursor solution. The reactions are rather complicated, but it is possible to hold them to two main types. One of them consists of hydrolysis, leading to the separation of alkoxide group(s) and production of silanols in accordance with a reaction,



where  $R$  is a hydrocarbon radical and  $n \leq 4$ . The condensation of monomers containing hydroxyl groups with each other



or with the initial precursor



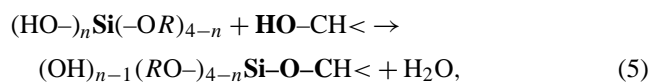
gives oligomers (oligosiloxanes) in which silicon atoms are linked through siloxane bonds. The oligomers form colloid particles of nanosize dimension (sol) in the bulk solution. It influences, but not strongly, the rheological behavior of solutions, which remains almost unchanged (Fig. 4). A sharp increase in the shear moduli occurs at the third stage owing to a sol–gel transition. It is caused by the cross-linking of previously formed sol particles containing hydroxyl groups on their surface generated by reaction (2). As a result, the linked nanoparticles turn out to be self-organized into a three-dimensional network consisting of the branched polysiloxanes. This is obvious from Fig. 13.

The addition of carrageenans into the precursor solution modified the sol–gel processes. The most important experimental facts are the shortening of the time interval for the gel setting and the gellification in the absence of common catalysts. Furthermore, polysaccharide macromolecules promote the mineralization, i.e., nucleation and growth of silicon compound. They manifest themselves as a template for the silica generated in the system.

It is worth mentioning that the acceleration of silica formation by biopolymers have been previously reported. It was first observed for peptides separated from diatom cell

walls [5,27,28]. A similar effect was then found by Stucky et al. [29,30] for synthetic cysteine–lysine block copolypeptides. The polysaccharides, such as cellulose [31] or alginate [32,33], influenced the morphology of synthesized silica, but they did not activate the sol–gel processes. This feature was first ascertained for carrageenans in this article.

Their effect is reasonable to relate to lots of hydroxyl groups in macromolecule (Fig. 1). The silanol monomers might condense with them, forming covalent bonds in accordance with a reaction:



where  $\text{HO}-\text{CH} <$  represents a hydroxyl group of a polysaccharide macromolecule. An alternative feasibility for the linking of silanols with carrageenans is in the formation of hydrogen bondings between hydroxyl groups. In both cases, a linked silanol molecule can further hydrolyze and then participate in the condensation reactions (3) and (4). This will lead to the formation of a silica shell around the polysaccharide chains (Fig. 14). In other words, carrageenans both promote mineralization in the mixed system and serve as the template for the generated inorganic component.

The formation of biomaterials of silica with carrageenans could be presented also by three stages: (i) hydrolysis of the precursor in accordance with reaction (2); (ii) condensation of the formed monomers on a polysaccharide macromolecule through reaction (5) or linkage with hydroxyl groups by the hydrogen bonds; (iii) polycondensation of the linked silanol molecules with silanol or precursor monomers according to reactions (3) and (4) to produce a silica shell surrounding the polysaccharide macromolecule.

The role of hydrogen bonds in the biomineralization was highlighted in various publications (see, for example, [5,34,35]). It is believed that they provide the association of silica with polysaccharide and protein macromolecules. Thus, the established acceleration of silica condensation by silicatein, a peptide separated from diatom cell walls, was explained by multiple hydroxyl group in this biopolymer [36]. The alternative opportunity, that is, the formation of covalent bonds between silicate and biopolymer macromolecules, has not been mentioned. The reaction with hydroxyl groups was observed for metal alkoxides mixed with ethyl cellulose [37].

The presented results on the carrageenan–silica biomaterials demonstrate a restriction on the conformational realignments of polysaccharide molecules owing to their mineralization. It is evident from an increase in the plateau modulus and yield stress of gelled hybrid materials (Figs. 8 and 9) as well as in the temperature corresponding to the phase transition (Figs. 11 and 12). Nonetheless, the carrageenan macromolecules are not strongly linked with the silica shell because they did not lose their ability for the reversible helix–coil transition. This fact argues for forming mainly the hydrogen bonds between carrageenan macromolecules and silicate. The covalent bonds in the case of their formation



in accordance with the condensation reaction (5) are of secondary importance.

The helix–coil transition experienced by carrageenans influences the mechanical properties of hybrid materials, but it does not determine them in a significant manner. There is only about a twofold decrease in the complex viscosity (Fig. 10A) when double helices melt, transferring into the coil state. This result signifies that the stability and stiffness of the three-dimensional network of gelled hybrid biomaterials is mainly governed by the silica framework.

It should be mentioned that the above-considered model is inconsistent with that suggested by Gill and Ballesteros [3,11]. They thought of interpenetrating networks formed by silica framework and biopolymers. The latter were not seen as a template for the inorganic component synthesizing in situ. The results presented in this article made it obvious that polysaccharides can promote mineralization processes in the mixed system, determining the structural organization of generated silicate at the nanoscale level (Figs. 13 and 14).

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