Review

Hybrid organic–inorganic nanocomposites fabricated with a novel biocompatible precursor using sol-gel processing

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Abstract—The synthesis of hybrid organic–inorganic nanocomposite materials containing biopolymers by the sol-gel processing is often faced with severe difficulties owing to their poor compatibility with the common precursors. The problem was solved when tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was recently suggested for the preparation of sol-gel derived nanocomposites. This article summarizes recent data on novel types of hybrid polysaccharide-silica nanocomposite materials fabricated with the help of THEOS. Their formation, properties and structure as well as features and possible mechanism for the sol-gel processes are considered. It is demonstrated that the novel approach is particularly suitable in situations where others are unsatisfactory. This includes systems of which supramolecular organization or phase state is sensitive to the pH of solutions, temperature and addition of organic solvents. One example of a successful application is the immobilization of labile enzymes. The biocompatibility of THEOS allows their activity to be retained, thereby enhancing long-term and thermal stabilities.

Keywords: Silica; polysaccharide; sol-gel; hydrogel; nanocomposite; mineralization; immobilization

1. INTRODUCTION

Organic–inorganic composite materials were prepared shortly after the synthetic polymers when the latter were mixed with inorganic components [1]. Now they present a great diversity of composites that have widespread applications in various areas. In recent years, attention was attracted to nanostructured hybrid materials owing to their unique and highly useful properties for optical, electronic and magnetic devices, catalytic materials, ceramics, adsorbents, membranes, biomaterials, coatings, etc. [2–15]. The organic–inorganic nanocomposites are usually fabricated by

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the sol-gel processes that are a subject of much current interest. The processing includes the use of a precursor, often metal or silicon alkoxides. When an alkoxide is mixed with water, it experiences hydrolysis and its products are involved in the condensation reactions leading first to a sol formation and then cross-linking of sol particles that causes the sol-gel transition [2, 11]. Silica-based hybrid materials have the most application.

There are two main approaches to fabricate the hybrid silica nanocomposites by the sol-gel technique. One is based on processes performed in media with self-organized structures of surfactants, block copolymers or polymers, while the other involves the use of organically modified silicone alkoxides. The resulting materials consist of the organic and inorganic components that may or may not have covalent bonds. This difference causes some distinctions in the structural organization and properties of hybrids nanocomposites. Generally, the synthesis with organically modified silicone alkoxides provides the control over the structure of fabricated materials at the molecular level, whereas the sol-gel processes carried out in the surfactant or polymer solutions can lead to nanocomposite materials with supramolecular organization. As examples of the former are inorganic–organic hybrid copolymers like ORMOCER®s [4] and of the latter, mesoporous silicates like FSM and MCM-families [16–18].

It is pertinent to note that hydrated silica is widespread in Nature and may be found in single-celled organisms to higher plants and animals [19, 20]. The biosilica is always found in associated forms with biopolymers and, as such, these complexes represent organic–inorganic composite materials. Moreover, the biosilica is built up, as shown, for example, in a case of diatom shells [21], from fibrils, tubular-like structures and particles ranging from a few nm to tens of nm, so they may justifiably be referred to as nanocomposites. The biopolymers are believed to control the silica nucleation and growth, thus regulating the size, structure, shape, spatial orientation and organization of biosilica [19, 20, 22, 23]. Glycoproteins, proteoglycans and polysaccharides are considered as the main nucleating and templating agents in the living cells [21].

The biomineralization processes feature high efficiency, while biominerals have superior mechanical properties in comparison with natural or synthesized inorganic counterparts [24–26]. This explains numerous studies devoted both to the understanding of the principles of tailor-made bioinorganics and their use for developing novel materials and devices with advanced structure and functions.

The principal role of proteins in biosilica synthesis was confirmed when silaffins [27, 28] and silicatein [29] were extracted from the diatom *Cylindrotheca fusiformis* and sponge *Tethya aurantia*, respectively, for experiments *in vitro*. The former promoted the silica precipitation from solutions of silicic acid, the latter, of tetraethoxysilane (TEOS). It is worth mentioning that the formation of silica nanoparticles was observed in both cases at ambient conditions. The proteins demonstrated themselves as structure-driven agents in the processing that is consistent with their role in living cells.

Attempts to manipulate sol-gel processes with the help of polysaccharides were not so successful. Experiments were performed with chitosan, alginate and cyclodextrins at conditions under which the silica gel was formed in their absence. The polysaccharides were entrapped into hybrid materials, but evidence of their influence on the processing was not reported. In systems included tetramethoxysilane (TMOS) and cyclodextrins, the precursor determined completely the conditions of processes.

TMOS and TEOS are common precursors that are currently applied to fabricate hybrid organic–inorganic composites. Generally, they are not highly compatible with biopolymers that causes difficulties for their use [8, 30, 31]. The limitations stem from the poor solubility of these precursors in water, which necessitates organic solvent addition, evolution of an alcohol in the course of the sol-gel processes and their performance at extreme pH or/and high temperature. All these factors bring about the precipitation and/or denaturation of biopolymers.

The problem of the compatibility of a silica precursor with polysaccharides was resolved when tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was applied, as in our recent work [32, 33]. The main advantage of THEOS over TMOS and TEOS, as demonstrated first by Hoffmann *et al.* [34, 35] (The authors synthesized THEOS by means of a procedure suggested by Mehrotra and Narain in 1967 [36]. By examining some properties of synthesized compounds, the latter considered their solubility in benzene. The behavior in aqueous solutions was not mentioned.), is complete water solubility that obviates the need for the organic solvent addition. Its amount may run to 50 wt% in a mixture with polysaccharides, and that does not lead to a phase separation [32, 33]. Homogeneous hybrid nanocomposites were prepared in a wide range of concentrations of both components. The compatibility is attributable to a special role of polysaccharides. They catalyze the sol-gel processes and serve as structure-driven agents. This role, demonstrated by us *in vitro* for the first time [37], is in line with the expectations that the polysaccharides may well provide silica nucleation and templating *in vivo*.

This article is devoted to our recent results on hybrid polysaccharide–silica nanocomposite materials fabricated with the help of THEOS. The features of sol-gel processes, properties and structure of materials, and a possible mechanism will be presented. It will also be demonstrated that the novel precursor provides new opportunities for developing advanced materials. THEOS allows us to carry out synthesis with surfactants of which supramolecular organization is sensitive to the pH of solutions, temperature and additions of organic solvents. Furthermore, the sol-gel processes under the polysaccharide control may also work in the presence of enzymes that allows preparation of biocatalysts with increased activity and long-term stability. These materials are very promising for biosensors and biotechnology.

2. CHEMICAL PROCESSES

The formation of silica nanocomposite materials may be represented by the following scheme:

$$\text{Precursor} + H_2O \xrightarrow{Hydrolysis} \text{Silanols} \xrightarrow{Condensation} \text{Sol} \xrightarrow{Cross\text{-}linking} \text{Gel}$$
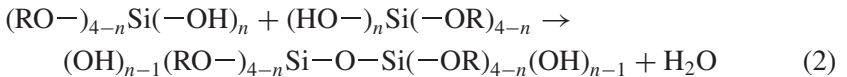
This scheme includes three main stages [2, 11, 38]: (1) hydrolysis of the alkoxide after the mixing with water; (2) condensation of the hydrolysis products (silanols) to produce oligomers arranged as sol particles; (3) cross-linking of the sol particles resulting in a transition into a gel state.

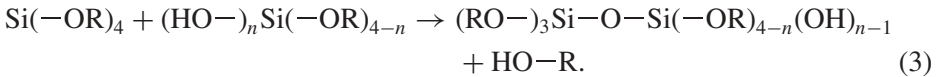The hydrolysis can be represented in general form by the reaction:

$$Si(-OR)_4 + nH_2O \rightarrow (HO-)_n Si(-OR)_{4-n} + nHO-R, \tag{1}$$
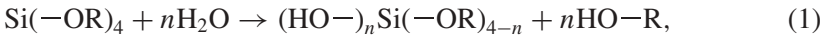
where R is a hydrocarbon radical and $n \leqslant 4$. The result is a silanol group ($Si-OH$) formed by the substitution of alkoxyl group to hydroxyl.

The condensation proceeds only through the mediation of a silanol group, but there are two main opportunities as shown by the following general reactions:

$$(RO-)_{4-n} Si(-OH)_n + (HO-)_n Si(-OR)_{4-n} \rightarrow$$
$$(OH)_{n-1}(RO-)_{4-n} Si-O-Si(-OR)_{4-n}(OH)_{n-1} + H_2O \tag{2}$$

and

$$Si(-OR)_4 + (HO-)_n Si(-OR)_{4-n} \rightarrow (RO-)_3 Si-O-Si(-OR)_{4-n}(OH)_{n-1}$$
$$+ HO-R. \tag{3}$$

The result in both cases is a dimer in which two silicon atoms are linked by a siloxane bond. The dimer may be involved in condensation reactions that lead to the formation of a trimer, then tetramer, and so on. However, the processing does not go further than the production of oligomers (oligosiloxanes). They are arranged as colloid particles of nanosize dimension (sol) in the bulk solution. The stability of the sol to the coagulation at near neutral pH is related to negative charge on the surface of particles [2, 11, 38].

The third stage, at which the sol-gel transition takes place, consists of the linkage of particles. This proceeds through their cross-linking by the condensation reactions (2) and (3); to promote it, it is usually necessary to introduce a catalyst. Since the sol stability to the coagulation is caused by the negative charge that is dependent on the pH, the processing is often triggered by the addition of acid or alkali into the solution [2, 11, 38]. Organic additives can also influence the particle aggregation that provides the basis for their manipulation on the sol-gel processes.

3. EFFECT OF POLYSACCHARIDES

A list of polysaccharides that influence the sol-gel processes with the participation of THEOS and which we studied is given in Table 1; they include anionic,

uncharged and cationic biopolymers. Charged groups are represented by carboxyl (alginate, xanthan, carboxymethylcellulose), sulfo (carrageenans and fucoidan), amino (chitosan) and ammonium (cationic derivatives of hydroxyethylcellulose, cat-HEC) groups. Most polysaccharides have a linear macromolecule; exceptions are represented by arabinogalactan, which is a branched polysaccharide, and α- and β-cyclodextrins, which are cyclic oligosaccharides consisting, respectively, of six and seven α $(1 \rightarrow 4)$-linked glucopyranose residues. Such polysaccharides as xanthan, locust bean gum, guar gum, hydroxyethylcellulose and its cationic derivatives have a linear backbone to which short chains are attached. Therefore, they are classed as graft-polymers. Alginate is a block copolymer of L-guluronic and D-mannuronic acids residues [39, 40]. Carbohydrate chains usually are not associated in solutions. This is observed in a case of xanthan [41, 42]. κ- and ι-carrageenans can form a double helix and experience a reversible coil-helix transition [43–45]. Owing to their propensity for association, xanthan, κ- and ι-carrageenans are capable of jellifying an aqueous solution, while the other polysaccharides can produce only a thickening effect.

A study of polysaccharides possessing various composition, structure and properties offered an understanding of the effect and role of this class of biopolymers in the sol-gel processing.
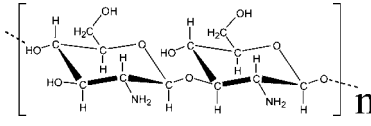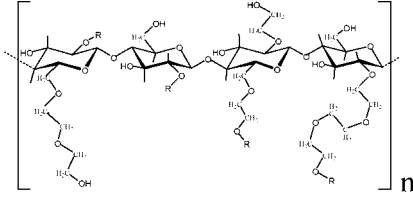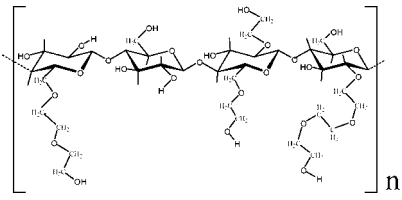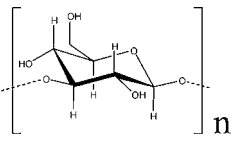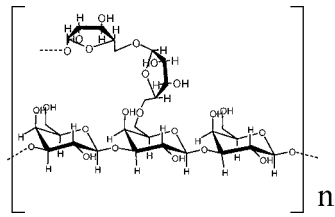
The effect of polysaccharides on the sol-gel processes was most pronounced at pH's near neutral. THEOS alone did not cause the jellification of water at ambient conditions over a period of a month. To trigger the sol-gel processes, it was necessary to add a catalyst, for example, an acid. When polysaccharides were admixed with THEOS at near neutral pH, the gel formation could be observed within a few minutes. Therefore, it was concluded that they exert a catalytic effect on the sol-gel processes [32, 33].

It is worth noting that polysaccharides are more efficient as catalysts than acids. The former after the addition produced the sol-gel transition within a few minutes; the latter required some 8–10 hours. Thus it is valid to say that the polysaccharides exert both accelerating and catalytic effects on the sol-gel processing with THEOS.

It is essential that the precursor was compatible with all the polysaccharides listed in Table 1 without exception. Their admixing in an aqueous solution was not accomplished with any phase separation or precipitation, even though the concentrations of THEOS and biopolymer could run, respectively, to 50 or only a few weight percent in the mixture. Furthermore, immediately the sol-gel transition happened, the solution remained homogeneous and optically transparent. This means that the silica generated *in situ* and the polysaccharides are also compatible. The further maturation of jelled materials proceeded in a variety of ways that depended on many factors, including the polysaccharide type and molecular weight, concentrations and concentration ratio between inorganic and organic components [32, 33]. Generally, opalescence developed with time, but the mixtures containing cationic polysaccharides remained transparent. In the presence of uncharged polysaccharides the hybrid materials became, as a rule, turbid or

Table 1.
Polysaccharides used to prepare hybrid silica nanocomposite materials

Polysaccharide	Structural formula	Type, MW (kDa)	Hydrogel
Cationic			
Chitosan		Linear, nd[a]	Opalescent, monolith
Cationic derivative of hydroxyethylcellulose (cat-HEC)	$R = CH_2\text{-}CHOH\text{-}CH_2\text{-}N^+(CH_3)_3\ Cl^-$	Graft polymer, 950	Transparent, monolith
Uncharged			
Hydroxyethyl-cellulose		Graft polymer, 950	Turbid, syneresis
Laminaran		Linear, 20-30	Turbid, syneresis
Arabinogalactan		Branched, nd	Turbid, syneresis

opaque. Furthermore, a syneresis, that is, a slow shrinkage of the gel volume accompanied by water release was observed. The hybrids that included charged polysaccharides usually remained as a monolith, being unchanged with time. It seems that the electrostatic repulsion between macromolecules exerted a stabilizing
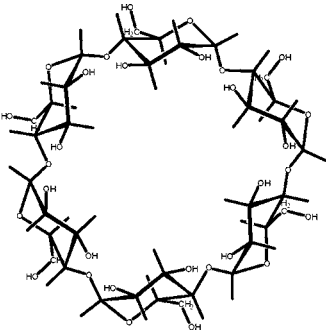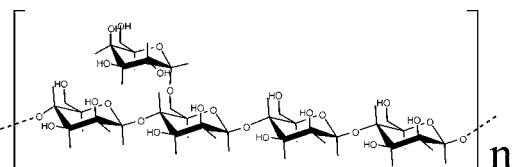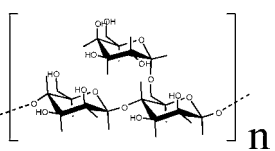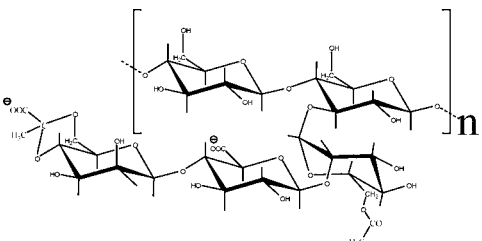
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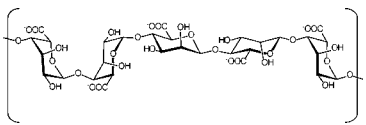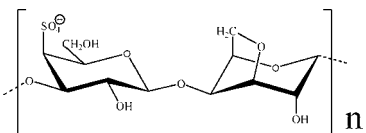
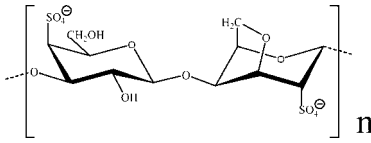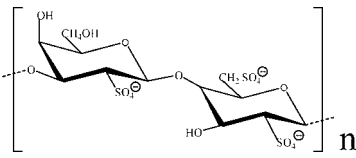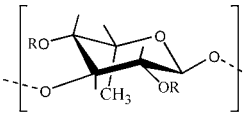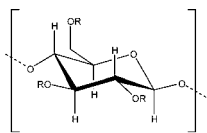| Polysaccharide	Structural formula	Type, MW (kDa)	Hydrogel
Alpha-cyclodextrin		Cyclic, 1394	Opalescent, monolith
Locust bean gum		Graft polymer, nd	Turbid, syneresis
Guar gum		Graft polymer, nd	Turbid, syneresis
Anionic			
Xanthan		Graft polymer, nd	Turbid, monolith
Alginate		Linear, block copolymer, 40	Turbid, monolith
Kappa-carrageenan		Linear, 700	Turbid, syneresis

Table 1.
(Continued)

Polysaccharide	Structural formula	Type, MW (kDa)	Hydrogel
Iota-carrageenan		700	Turbid, monolith
Lambda-carrageenan		1020	Turbid, monolith
Fucoidan	$R = H \text{ or } SO_3^-$	Linear, 20-35	Turbid, monolith
Carboxy-methyl-cellulose	$R = H \text{ or } CH_2COO^-$	Linear, 360	Opalescent, monolith

[a] nd, not determined.

effect on the hybrids against shrinkage, although it did not always take place. As an example is the hybrid material with κ-carrageenan [32]. Water separated from the initially synthesized monolith in the next days after the preparation. The shrinkage, which continued for 5–7 days, resembled the syneresis that developed in the hydrogels of κ-carrageenan alone (see, for example, Ref. [46]). This example demonstrates that polysaccharides retain their properties in the hybrid materials.

The accelerating and catalytic effects of polysaccharides on the sol-gel processing with THEOS were not mentioned where experiments were carried out with TMOS or TEOS and sodium silicate in aqueous solutions. A case in point is the synthesis of nanocomposites incorporating cyclodextrins. As the precursors, TMOS and TEOS were applied. The processing was performed at conditions at which these silanes form gel in the absence of polysaccharide, It included an acidification of solutions, addition of an alcohol [47] or removal of that produced in the course of processing to prevent the cyclodextrin precipitation [48, 49]. When sodium silicate was involved in the synthesis, it was also necessary at least to add an acid to trigger the gel setting in the presence of alginate [50, 51] or perform the processing at increased temperature and pressure to have hybrid chitosan-silica materials [52].

A system resembling THEOS, but more complicated was suggested by Gill and Ballesteros [30]. It involved polyol esters of oligosilicates. They were prepared by a transesterificaion of the oligomer products of partial hydrolysis and condensation of TEOS to exchange ethanol to various polyols. The system should be regarded as a silica sol. It was easily transferred to the gel at near neutral pH even in the absence a catalyst. The best results were obtained with poly(glyceryl silicate). It is pertinent to note that any effect of biopolymers on the sol-gel transition was not mentioned. The authors only particularly emphasized the biocompatibility of their poly(glyceryl silicate) [30, 31]. This is also typical of THEOS.

4. PROPERTIES OF HYBRID POLYSACCHARIDE-SILICA NANOCOMPOSITES

The material fabricated as a result of the sol-gel processing with THEOS represents a gel that includes water and ethylene glycol as solvents. The latter is evolved in the course of the precursor hydrolysis (reaction (1)). Where the initial concentration of THEOS did not exceed 10 wt%, the sol-gel derived materials may be classed as hydrogels because the main solvent is water there (as much as 90 wt%). When the precursor amount is increased, there is more ethylene glycol in the jelled material. The fabricated nanocomposites could contain as much as 50 wt% of the organic solvent. It is reasonable to refer to them as a mixed system. An important point is that ethylene glycol even at increased concentrations is completely compatible with polysaccharides. Therefore, phase separation or precipitation was not observed [32, 33, 53].

The mechanical properties of gels are of prime importance for their various applications. They were studied in some detail in our earlier work [32, 33]. To illustrate their dependence on the content of polysaccharide and silicate in the hybrid materials, Figs 1 and 2 present the main rheological parameters — zero shear viscosity η_o, plateau modulus G_o, yield stress σ_y and critical strain γ_{cr} corresponding to the yield stress — *versus* the concentrations of, respectively, xanthan and THEOS in the initial solution. It is obvious at a glance that the increase of amounts of polysaccharide and silicate, when the content of counterpart component remained unchanged, has opposite effects on the mechanical properties of the hydrogels. The former provides a decrease in the mechanical strength, making them softer, which follows from the concentration dependencies of zero-shear viscosity, plateau modulus and yield stress (Figs 1A, 1B and 1C). This is accompanied by an increase in the hydrogel elasticity. The effect is evident from an increase of critical strain (Fig. 1D) at which gel flowing or breaking commences under the action of an applied shear stress equal to the yield stress value [32]. The zero-shear viscosity, plateau modulus and yield stress varies almost inversely with the polysaccharide concentration. The power law exponents found by the best linear fit to the data for η_o, G_o and σ_y are equal to 1.1, 1.4 and 1.4, respectively. The elasticity increases with the xanthan concentration, though not in such a large measure. The power law exponent is 0.17.
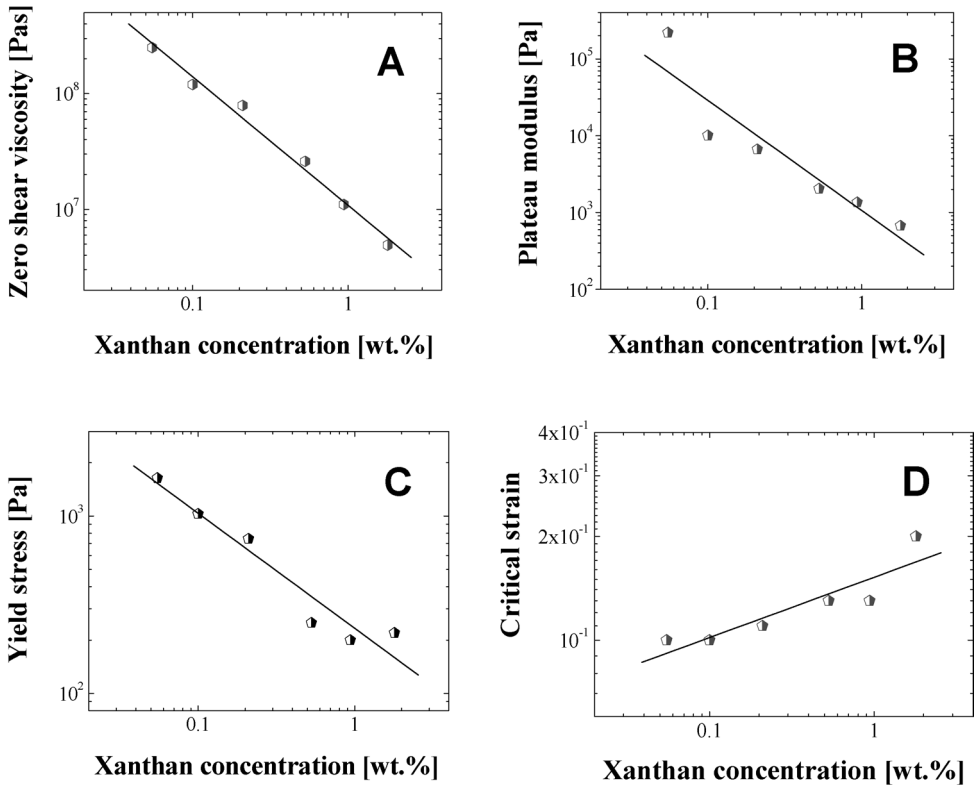
Figure 1. The zero shear viscosity (A), plateau modulus (B), yield stress (C) and critical strain corresponding σ_y (D) *vs.* the concentration of xanthan (A). The hydrogels were synthesized by introducing of 10 wt%. THEOS. Rheological measurements were performed at 25°C.

The silica, as obvious from Figs 2A–D, made the hydrogel stiffer and more brittle. It is significant that the effect of inorganic component is more pronounced than that of polysaccharides. Values of power law exponents found from the concentration dependencies for zero-shear viscosity, plateau modulus and yield stress in Figs 2A, 2B and 2C are 3.2, 2.7 and 1.3, respectively. The elasticity also changed more significantly than was found in Fig. 1D. The critical strain (Fig. 2D) is proportional to the inverse 0.77 power of THEOS content in the initial solution.

The plateau modulus in the classical theory of rubber-like elasticity is proportional to the number of cross-links ν in accordance with the equation [54]:

$$G_o = \nu A k T, \tag{4}$$

where k and T are the Boltzmann constant and the absolute temperature, respectively, and A is a numerical factor close to unity. When the dependence of G_o on the concentration of THEOS is examined (Fig. 2B), the number ν can be formally equated with the linkages formed between silicon atoms as result of the condensation reactions (2) and (3). In such a case, a value of 2.7 for the power law exponent
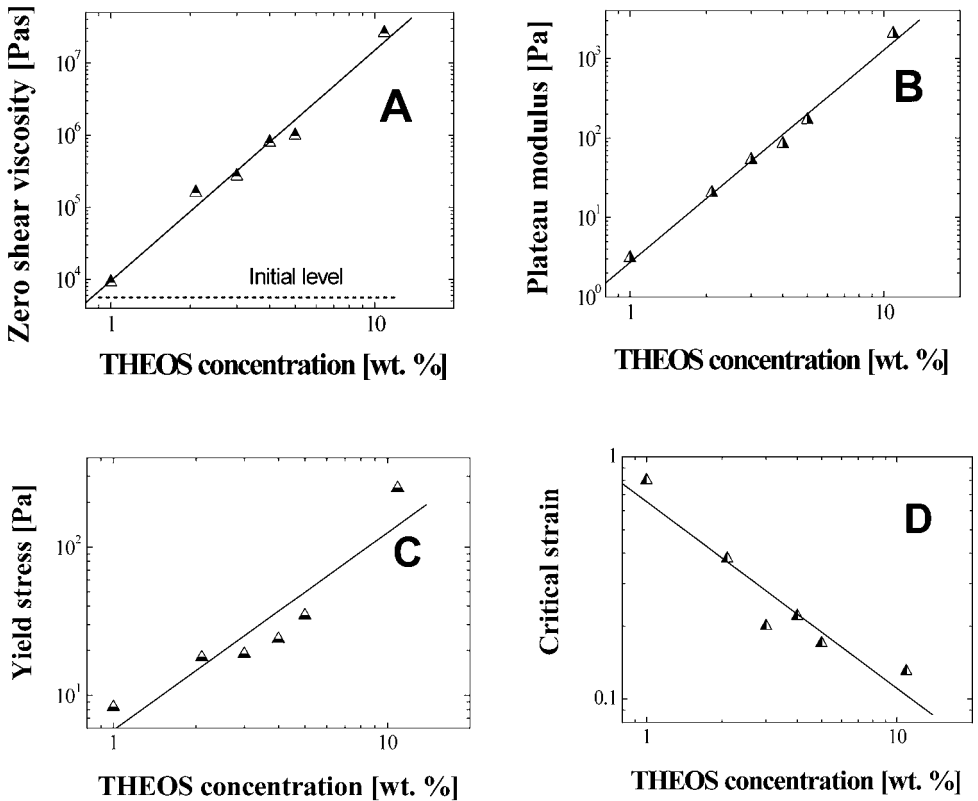
Figure 2. The zero shear viscosity (A), plateau modulus (B), yield stress (C) and critical strain corresponding σ_y (D) *vs.* the concentration of THEOS in the initial solution. The concentration of xanthan was 0.5 wt%. The dashed line marks the zero shear viscosity of solution containing polysaccharide alone.

followed from the experimental dependence in Fig. 2B, indicating that the silica in the examined hydrogel is moderately branched. For the densest material, the theoretically possible ν value could be as much as 4.

It is important to note that the scaling relationships similar to those in Fig. 2 vary from polysaccharide to polysaccharide. Furthermore, they are dependent on the polysaccharide concentration in the hydrogel. For example, the values of the power law exponents in a case of the plateau modulus were decreased from 3.8 to 2 when the xanthan content was increased from 0.25 to 1 wt%. This points to an influence of polysaccharides on the condensation reactions leading to the silica generation by the sol-gel processing. The reason for the polysaccharide effect is a formation of hydrogen bonds between a silanol group and hydroxyl group of carbohydrate macromolecules, but to date this has not been studied in sufficient detail.

The effect of silica, which is generated *in situ* by the sol-gel processing in an initial polysaccharide solution, becomes apparent when its concentration reaches a threshold level. In the considered case (Figs 2A–D), a strengthening of xanthan

hydrogels was observed where the concentration of THEOS in the solution was larger than 1 wt%. The initial level observed before the silica formation is represented by a dashed straight line. At smaller precursor content (<1 wt%), the rheological characteristics of the hydrogel were not different from those measured in the presence of xanthan alone. In turn, the polysaccharides had an effect on the mechanical properties at smaller concentrations than silica. Xanthan, which is the most effective organic component among them, began its influence at a concentration of 0.05 wt% (Figs 1A–D). The effect of polysaccharides usually appeared when their amount in the hydrogel was as much as 0.2 wt%.

5. STRUCTURE OF HYBRID MATERIALS

Figure 3 presents SEM pictures of hydrogels. They are arranged into two rows of which the upper shows pictures obtained at low magnification, the bottom, at larger. This enables the structural organization of the same hydrogels to be seen at two levels. The samples were prepared with three different polysaccharides, (from left to right) chitosan, cat-HEC and xanthan, but their concentration and silica content were identical.

It is obvious from these photographs that the main morphological elements of hydrogels are a cross-linked network and spherical particles. The former forms a framework of material, while the latter fill the mesh space. The spherical particles are of secondary structural importance because the connections (cross-links) between them are not evident in most cases. They and the network are made up of silicate.

The morphological features of hydrogels, as follows from a comparison of the photographs in Fig. 3, vary when the polysaccharide used in the sol-gel synthesis is changed. This shows up both in the network density and dimensions of spherical particles. The observed differences mean that the structure of hybrid materials depends on the nature of the carbohydrate. It was also demonstrated in Shchipunov and Karpenko [33] that the charge density on macromolecules and biopolymer concentration in the initial solution also had a significant effect. Inasmuch as the jellification time also varies with type of polysaccharide, it is believed that the kinetics of reactions (1)–(3) in the sol-gel processes is regulated by the biopolymer. To date this is only a speculation because the processing mechanism has not been studied.

Figure 4 presents an SEM photograph of an aerogel. The initial hydrogel was synthesized by mixing 0.5 wt% of κ-carrageenan and 50 wt% of THEOS: cross-linked fibrils forming a network can clearly be seen. This morphology is consistent with that found in the hydrogels (Fig. 3), although there are notable differences in details. In the first place, the fibrils differ in their thickness, being much thinner in the aerogel. This may be caused by the increased concentration of THEOS. The higher is the content of precursor in the initial solution, the faster is the jellification. The change is probably caused by a variation in the ratio between the kinetics of
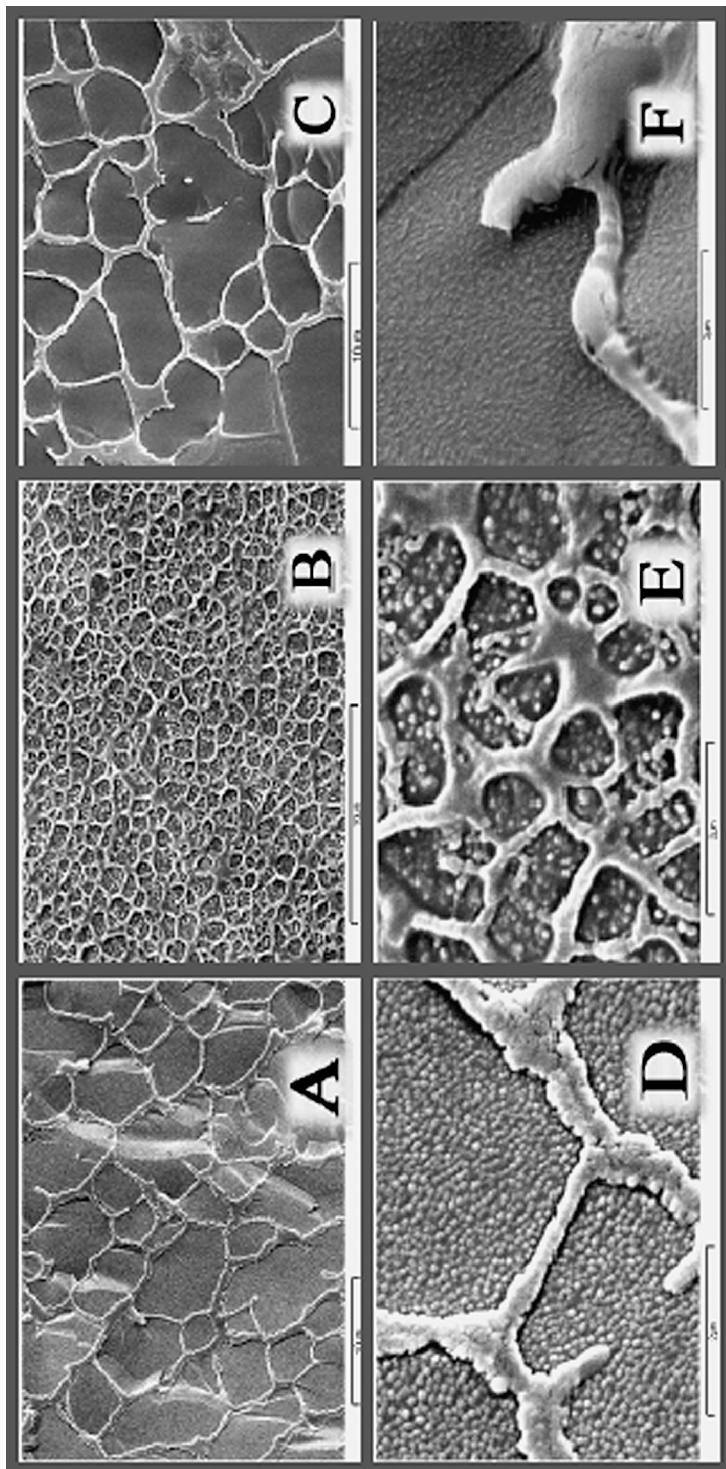
Figure 3. SEM micrographs of hydrogels synthesized in an aqueous solution of chitosan (A, D), cat-HEC (B, E) and xanthan (C, F). The concentrations of polysaccharides and THEOS were 1 and 10 wt%, respectively.
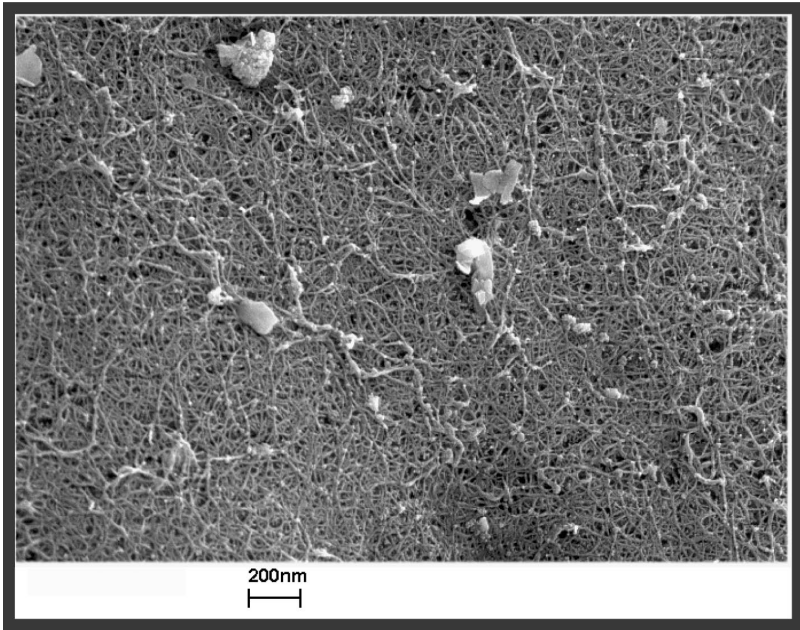
Figure 4. SEM micrographs of aerogel that was prepared from a hydrogel synthesized in an aqueous solution containing 50 wt% THEOS and 0.5 wt% of κ-carrageenan.

hydrolysis and condensation (reactions (1) and (2) or (3), respectively). When the former prevails over the latter, the sol-gel processing leads, as is well known [2, 11], to the formation of finer silica particles. In the second place, the spherical particles are not found in the aerogel. There are only a few silica particles, but they are superior to the fibrils and mesh space. It is conceivable that smaller particles could be washed out when water was substituted for acetone during the preparation of sample to the supercritical drying.

The fibrillar structure is typical of hybrid materials fabricated by using linear, grafted or branched polysaccharides (Table 1). An essential difference was found when cyclodextrins were used to synthesize nanocomposites by the sol-gel process-ing. Figure 5A demonstrates an AFM image of a xerogel that was prepared by calcination of a hydrogel containing 5 wt% of α-cyclodextrin at 500°C. Numerous dark circular spots about 0.7 nm in diameter may be seen. They were not observed when synthesis was performed in the presence of trace amounts of cyclodextrin that was added only to catalyze the sol-gel processes. An image of this xerogel showing a homogeneous surface is shown in Fig. 5B. The absence of pores, which are typical of this type of material, was caused by their collapse in the course of hydrogel cal-cination. A comparison of the pictures presented in Fig. 5 enabled us to suggest that the dark spots represent spherical pores formed in the place of carbohydrate macro-molecules. Their diameter is about two times smaller than the outer diameter of α-cyclodextrin (1.37 nm [55]). The smaller dimension is explained by a significant shrinkage of the pores during the calcining procedure.
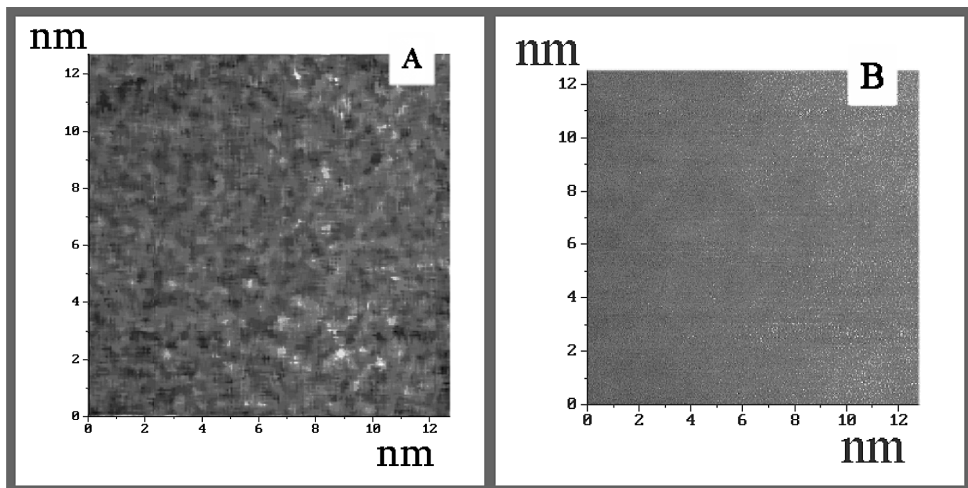
Figure 5. (A) AFM images of xerogels that were prepared by calcination of hydrogels initially synthsized in an aqueous solution containing 10 wt% of THEOS and 5 wt% (A) or trace amounts (B) of α-cyclodextrin.

It should be mentioned that the cyclodextrin-containing hybrid materials were also synthesized by applying TMOS and TEOS [47, 48, 56, 57]. The procedures were more complicated than that performed with THEOS. For example, the sol-gel processing in [48, 56] was carried out under reduced pressure to remove methanol evolved in the course of TMOS hydrolysis. The pores were about three times greater in diameter than those seen in Fig. 5A. A better agreement in our estimation is with the results of work by Wallington *et al.* [57]. The smallest pores in their synthesized materials were 0.5–0.7 nm in diameter, but the nanocomposite was inhomogeneous. There were also pores with diameters of 7–14 and 25–75 nm.

6. MECHANISM OF SOL-GEL PROCESSES

A reason for the catalytic effect of polysaccharides on the sol-gel processing was considered in our earlier paper [33]. By adding high-molecular weight poly(ethylene oxide) (PEO) or poly(vinyl paperalcohol) (PVA) in a THEOS-containing solution, it was found that a gel settled only in the presence of PVA. This experiment showed the importance of hydroxyl groups, because PEO contained a negligible number of terminal HO groups in distinction to PVA of which macromolecules had a hydroxyl group attached to almost every monomer residue. As polyhydroxy compounds are composed of numerous monosaccharide residues (Table 1), the polysaccharides are capable of forming hydrogen bonds with silanols produced in the course of hydrolysis of the precursor (reaction (1)). This can bring about silica nucleation on the macromolecules.

The nucleation means that the polysaccharides could be a template for the silica generated *in situ* in the course of sol-gel processes. To provide evidence for

this suggestion, an AFM was applied in [37]. A schematic representation of experimental procedure used to prepare a sample for the observation is given in Fig. 6. In an early stage, a mica surface was covered by a monolayer of arachidic acid placed by the Langmuir-Blodgett technique. It was done because the substrate surface was not inert, causing silica precipitation from a solution. The acid molecules were oriented outward by the hydrocarbon chains that excluded the sol-gel processing.

The subsequent stage consisted in adsorbing a hydrophobically modified cat-HEC from a highly diluted aqueous solution to have separated carbohydrate macromolecules on the acid monolayer. It was followed by a treatment in a precursor solution for various times. An examination of thus prepared samples by us [37] demonstrated that an initial nucleation and subsequent grow of silica precipitate proceeded on the cat-HEC macromolecules. Figure 7 shows an image of a mica surface at an intermediate stage of the treatment. One may distinguish carbohydrate macromolecules covered by silica that resemble icebergs on the water surface. The latter presents the mica surface filled by an acid monolayer. With the passage of time, the macromolecules are overgrown with silica that spread over the surface, merging with neighboring silica precipitates to the point where, as shown in [37], it resembled the network structure found in the case of polysaccharide-silica hydrogels (Fig. 3). The resemblance between the two-dimensionally and three-dimensionally generated morphology suggests that the sol-gel processes in the vicinity of mica surface and in the solution bulk are not notably different, and that the carbohydrate macromolecules serve as the template for the silica in both cases. This observation gives good reason to consider that the formation of network structure in Fig. 3 is the result of silica nucleation and subsequent growth on a macromolecule that leads to the generation of an inorganic shell around it.

The considered mechanism differs from that of Gill and Ballesteros [30, 31]. They suggested an interpenetrating network formed by polysaccharides and silicate in the jelled material. This could be the case of non-interacting components, that is, where the polysaccharide is neutral to the products of silane hydrolysis and silica generated in the course of sol-gel processes. In such a situation, the carbohydrate macromolecules would be entrapped into the silica matrix. The hybrid polysaccharide-silica materials synthesized by applying THEOS present the case of interacting components.

7. FUTURE DIRECTIONS

The considered approach for the fabrication of hybrid silica materials is not restricted to just polysaccharides. It became obvious when our experimental results provided an insight into the mechanism of sol-gel processing. Because the catalytic and templating effects are related to the hydrogen bonding between polysaccharide and silica, an idea of using other substances liable to formation of H-bonds was conceived. It was tested in the case of an N-dodecanoyl-β-alanine [58].
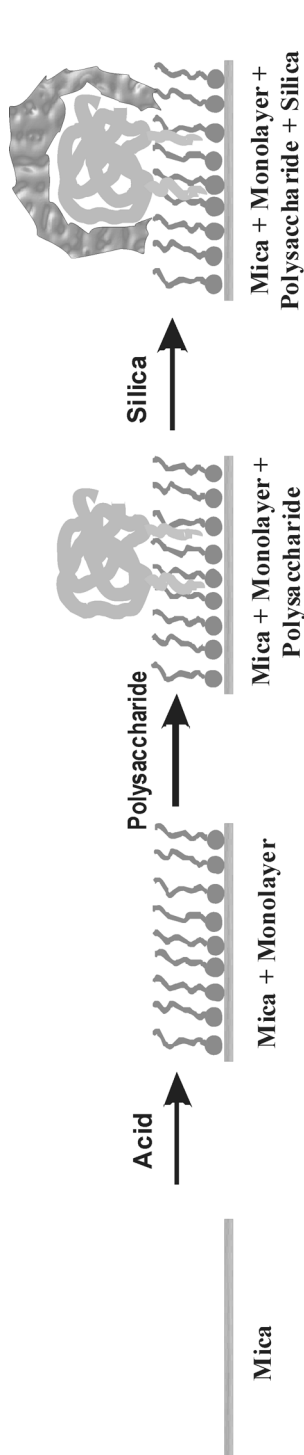
Figure 6. A schematic representation of the experimental procedure to observe the nucleation of silica on carbohydrate macromolecules. It includes the coating of mica surface by a LB monomolecular film of arachidic acid, an adsorption of hydrophobically modified cat-HEC on it and an exposure in a THEOS solution.
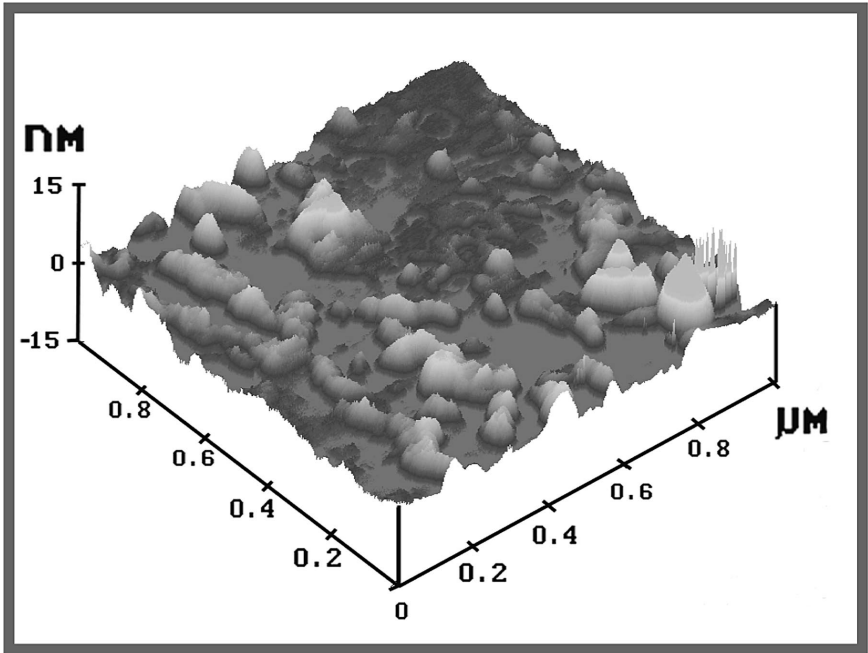
Figure 7. An AFM image of a sample prepared in accordance with the procedure shown in Fig. 6.

This surfactant with an amino acid residue can self-assemble into fibrils in aqueous solutions [59]. Its peculiarity is that fibrous aggregates are formed at pH around 6 and temperature not exceeding 25 °C. These are inappropriate conditions to perform sol-gel processes with a common precursor, if one decided to use surfactant fibrils as a template for silica. The shift of pH or increase of temperature resulted in the disintegration of fibrous aggregates when an attempt was made to apply TEOS [58]. Fibrils covered by a silica shell could be prepared when THEOS was added into a solution with alanine-containing surfactant. The sol-gel processing proceeded without the addition of a catalyst or heating because the catalytic and templating role was fulfilled by amino acid residues located on the fibrous surface. This is explained by their well-known propensity to form hydrogen bonds.

The above-considered example shows that the main advantage of THEOS over common precursors is in that it can be used at conditions at which others do not work. THEOS is useful where the phase state of a system is strongly dependent on the pH and temperature. For this reason it can be combined with biopolymers. Proteins provide an additional example. Because of large amount of polar groups that are capable of forming hydrogen bonds, they can also catalyze the sol-gel processes. We have verified this when THEOS was added into a solution with bovine serum albumin, caseins or gelatin. This resulted in the preparation of hybrid protein-silica nanocomposite materials. Their synthesis and properties resembled those for polysaccharides considered in this article, but this has not been studied in

sufficient detail yet. A study on the protein-containing materials presents a future direction in this considerably promising field.

The sol-gel technology with THEOS is of profound interest for the immobilization of enzymes. The majority of them are usually functioning at near-neutral pH. Acidification or alkalization as well as heating of solutions cause protein denaturation. We found that THEOS as precursor is ideally suited for enzyme entrapment [53, 60]. Its main advantage over current precursors lies in the fact that the entrapment conditions are dictated by the enzyme, but not the sol-gel processing. This enabled us to immobilize a very labile protein that loses its activity in solution even at appropriate conditions within a few hours [60]. After the entrapment into the silica matrix, it was active for a few months. The long-term stability was supplemented by increased thermostability. The notable stabilization of enzymes in the immobilized state has considerable promise for development of biocatalysts for biosensors and biotechnology.

Acknowledgements

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