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Journal of Colloid and Interface Science ●●● (●●●●) ●●●-●●●

JOURNAL OF  
Colloid and  
Interface Science

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Priority communication

# Gelling of otherwise nongelable polysaccharides

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Received 27 December 2004; accepted 2 February 2005

Vol. 287, No. 2, p. 373-378

## Abstract

It was shown first that solutions of nongelable polysaccharides, including such practically important ones as chitosan, hyaluronate, and cyclodextrin, can be gelled by generating silica in situ via sol-gel processing. This could be done owing to a novel silica precursor that is completely water-soluble and compatible with biopolymers. The gelation was caused by mineralization of carbohydrate macromolecules, which strengthened them and provided their cross-linking.

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**Keywords:** Sol-gel; Chitosan; Hyaluronate; Cyclodextrin; Mineralization; Silica nanocomposite; Organic-inorganic nanocomposite; Hydrogel; Biomaterial

## 1. Introduction

Polysaccharides are widely used as gelling agents in the food, pharmaceutical, cosmetics, and other industries [1-5]. However, this group is not so ~~useful~~ as one would like. Most polysaccharides increase only the viscosity, not forming hydrogels. There are numerous attempts to transfer them into the gelling group using various approaches. The general practice is to use a cross-linking procedure or modify their macromolecules chemically, as in the case, for example, of such practically important polysaccharides as chitosan or hyaluronate [6-10]. Cross-linking of macromolecules or chemical modification enables one to reach gelation, but the hydrogel biocompatibility of these biopolymers is heavily deteriorated.

Here we demonstrate first that solutions of nongelable polysaccharide can be gelled by mineralizing their macromolecules with silica. This is a completely biocompatible inorganic material because hydrated silica is widespread in living nature, from single-celled organisms to higher plants and animals [11,12]. As a rule it is associated with biopolymers. The latter are believed to control silica nucleation and growth (biomineralization), thus regulating the size, struc-

ture, shape, spatial orientation, and organization of biosilica [11-14]. Glycoproteins, proteoglycans, and polysaccharides are considered to be the main nucleating and templating agents in living cells [15]. Silica formation is due to the sol-gel processing caused by polycondensation of silicic acid. Processes resembling biomineralization are suggested to apply in this article, but instead of silicic acid, tetrakis(2-hydroxyethyl) orthosilicate (THEOS) is used. This is a novel silica precursor introduced in Refs. [16,17]. It hydrolyzes, producing a silicic acid, when it is added to an aqueous solution. An advantage of THEOS over currently used tetramethoxy- and tetraethoxysilane (TMOS and TEOS, respectively) is complete compatibility with biopolymers [18-20]. As shown here, this enabled us to perform sol-gel processes in solutions that resulted in the gelation of otherwise nongelable polysaccharides.

## 2. Experimental

### 2.1. Materials

Chitosan, guar gum, locust bean gum, and carboxymethylcellulose were purchased from Fluka, alpha-cyclodextrin from Wacker (Germany), and beta-cyclodextrin from ICN (USA). Sodium hyaluronate was a gift from Dr. T. Yanaki

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(Shiseido, Japan). Fucoïdan and laminaran were presented by Professor T.N. Zvyagintseva (Vladivostok, Russia), arabinogalactan by Dr. (Irkutsk, Russia). Hydroxyethylcellulose (950 kDa) and its cationic derivatives cat-HEC (950 kDa) were obtained from Hoekst (Germany). The polysaccharides were used as received. Their list is given in Table 1, where their structural formulas are also shown.

The silica precursor tetrakis(2-hydroxyethyl) orthosilicate (THEOS) was synthesized from tetraethoxysilane (ABCR, Germany) as described in detail in [21].

## 2.2. Methods

### 2.2.1. Hydrogel preparation

An aqueous solution of polysaccharide with pH between 5.5 and 6.0 was initially prepared by dissolving it in water. It was left for 1 day. An appropriate weighed amount of silica precursor was added into the polysaccharide solution and thoroughly stirred. The mixture was set aside at ambient temperature at least for a week prior to examination of its properties. This period of time was enough to have a hydrogel with reproducible rheological properties.

### 2.2.2. Rheological measurements

Rheological measurements were performed with a Rotovisco RT20 stress-controlled rheometer (Haake) by using cells having the cone-and-plate geometry of two versions. Low-viscosity mixtures were examined in a cell with the double gap, hydrogels in a cell with a common single gap. The cone angle was 1° and its diameter 50 mm. The oscillatory shear measurements were made when the frequency was changed from 0.001 to 10 Hz. The zero-shear viscosity  $\eta_0$  for liquidlike mixtures was found from a dependence of the complex viscosity against frequency  $f$  at  $f \rightarrow 0$ , for hydrogels with solidlike behavior, from the creep measurements as described in [18]. The creep technique was also used to determine the plateau modulus  $G_0$ . The yield stress value  $\sigma_y$  was found from stress sweep measurements [18].

### 2.2.3. Scanning electron microscopy

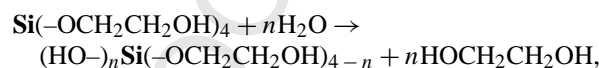
A sample for observation was prepared by freezing a polysaccharide–silica hydrogel with liquid nitrogen. Then it was cleft to have a fresh surface that was covered by an evaporated platinum layer. Micrographs were taken by an FE-SEM Leo 1530 electron microscope.

## 3. Results and discussion

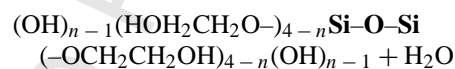
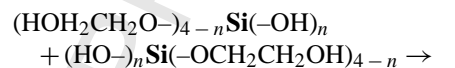
Polysaccharides studied in this work are listed in Table 1. They were of various types. The macromolecules were uncharged when they contained only hydroxyl groups, or charged when they bore additionally anionic or cationic functional groups. Most polysaccharides had a linear backbone, but there were also arabinogalactan and  $\alpha$ - and  $\beta$ -cyclodextrins, which were respectively branched and cyclic

biopolymers. Such polysaccharides as locust bean gum, guar gum, hydroxyethylcellulose (HEC), and its cationic derivative (cat-HEC) are referred to as graft-polymers owing to short chains attached to the linear backbones. In spite of their structural diversity, all the polysaccharides mentioned may be assigned to a particular group of biopolymers that are unable to transform aqueous solutions into a gel state. They could only increase the viscosity, providing a thickening effect.

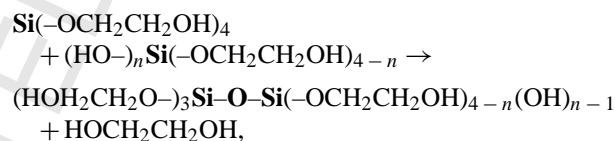
A transition into a gel state was found in the polysaccharide solutions only when THEOS was introduced. This is a silica precursor that is hydrolyzed after contact with water in accordance with the reaction



where  $\text{HOCH}_2\text{CH}_2\text{OH}$  is ethylene glycol and  $n \leq 4$ . The formed silanol group(s) ( $\text{Si}-\text{OH}$ ) in the hydrolyzed molecules are liable to condensation reactions,



and



which are at the root of sol–gel processes and formation of silica nanocomposite materials [22,23].

The introduction of THEOS induced the gelation of polysaccharide solutions, in general, at a concentration beginning from a few wt%. It should be stressed that the silica precursor was completely compatible with biopolymers. Phase separation or precipitation was not observed after their mixing. The gelled solutions were also homogeneous. Their optical properties depended on the polysaccharide type and concentration. These are mentioned in Table 1. The hydrogels were stable in time. Phase separation and change in the optical properties were not observed within a few months or, in some specially examined cases, within a year.

To carry out the sol–gel processes in the presence of THEOS, it was unnecessary to introduce a catalyst, although they took place at circumneutral pH. This is caused, as shown previously in [18,19], by a catalytic effect of the polysaccharides.

It is worth mentioning advantages of THEOS as a precursor over the currently used TMOS and TEOS or sodium silicate. It enabled us to transform solutions of nongelable polysaccharides into the gel state. The common precursors, as discussed in Refs. [18–20], are not sufficiently compatible with biopolymers. This is caused by introducing an acid or alkali to catalyze the processes and an organic solvent, usually an alcohol, to provide the solubility of TMOS and

Table 1

Nonjellified polysaccharides used to prepare a hydrogel; their structure and type as well as sol-gel transition concentration and hydrogel characteristics

Polysaccharide	Structural formula	Type	$C_{s-g}^a$ (wt%)	Hydrogel
Chitosan		Linear, cationic	nd <sup>b</sup>	Opalescent, monolith
Cationic derivative of hydroxyethylcellulose (cat-HEC)		Graft polymer, cationic	0.5	Transparent, monolith
Hydroxyethylcellulose (HEC)		Graft polymer, uncharged	nd	Turbid, syneresis
Laminaran		Linear, uncharged	nd	Turbid, syneresis
Arabinogalactan		Branched, uncharged	~5	Turbid, syneresis
Alpha- and beta-cyclodextrin		Cyclic, uncharged	~4	Opalescent, monolith
Locust bean gum		Graft polymer, uncharged	~5	Turbid, monolith
Guar gum		Graft polymer, uncharged	nd	Turbid, syneresis
Fucoidan		Linear, anionic	nd	Turbid, monolith
Carboxymethylcellulose		Linear, anionic	nd	Opalescent, monolith
Sodium hyaluronate		Linear, anionic	2	Opalescent, monolith

<sup>a</sup> Concentration of the silica precursor at which the sol-gel transition happens.<sup>b</sup> Not determined.

TEOS, but it precipitates most polysaccharides. In addition, an alcohol is evolved in the course of precursor hydrolysis. Ethylene glycol separated by THEOS does not so notably influence the biopolymer solubility. However, successful at-

tempts to fabricate hybrid polysaccharide-silica nanocomposite materials were undertaken in a few labs using TMOS, TEOS, and sodium silicate. To our knowledge, synthesis has been performed with cyclodextrins [24-26], chitosan [27],

1 and alginate [28,29], but the latter refers to gelable polysac-  
2 charides that are outside the subject of article. A case in point  
3 are cyclodextrins and chitosan, whose are mentioned in Ta-  
4 ble 1. The sol–gel processes were carried out at conditions at  
5 which TMOS, TEOS, and sodium silicate formed a gel in the  
6 absence of polysaccharides. It included acidification of solu-  
7 tions, addition of an alcohol, or removal of that produced in  
8 the course of precursor hydrolysis to prevent cyclodextrin  
9 precipitation. Evidence of the accelerating or catalytic influ-  
10 ence of polysaccharides on processing with TMOS, TEOS,  
11 and sodium silicate was not reported. Moreover, the synthe-  
12 sis was made, as in the case of chitosan and  $\text{Na}_2\text{SiO}_3$ , at  
13 increased temperature and pressure. It is also reasonable to  
14 point out that the amount of silica precursors involved in the  
15 synthesis of hybrid materials was at the level of 30 wt%.

16 A system resembling THEOS, but not a precursor in the  
17 commonly accepted sense, was suggested in [30]. It con-  
18 sisted of a silica sol from polyol esters of oligosilicates that  
19 were prepared by transesterification of the oligomer prod-  
20 ucts of partial hydrolysis and condensation of TEOS to ex-  
21 change ethanol to various polyols. The authors could fab-  
22 ricate polysaccharide-containing hydrogels at circumneutral  
23 pH and ambient conditions even in the absence of a catalyst,  
24 but an effect of biopolymers on sol–gel processing was not  
25 mentioned [30,31].

26 THEOS addition into solutions of polysaccharides shown  
27 in Table 1 induced their transition into a gel state when the  
28 precursor concentration reached a critical level. This fol-  
29 lows from rheological measurements. Fig. 1 presents a set  
30 of dependences of the shear moduli (storage moduli  $G'$  and  
31 loss moduli  $G''$ ) against the oscillation frequency of applied  
32 shear stress for three systems. One, represented by curves 1  
33 and 2, was a 1.5 wt% aqueous solution of cat-HEC. The  
34 curves corresponding to the storage moduli  $G'$  (curve 1) and  
35 loss moduli  $G''$  (curve 2) are placed one below the other. The  
36 shear moduli scale as  $G' \sim f^2$  and  $G'' \sim f$ . This type of  
37 dependence is characteristic of liquids [32,33]. The polysac-  
38 charide macromolecules in the solution interact with each  
39 other, but the interactions are physical in their nature (mainly  
40 van der Waals and electrostatic ones). There are only tempo-  
41 rary contacts between them. Covalent cross-links are absent.

42 The rest of the data in Fig. 1 refer to systems containing  
43 silica. It was produced with the help of sol–gel processing  
44 by introducing THEOS into a 1.5 wt% cat-HEC solution. As  
45 seen, the curves are shifted up with introduction of 0.5 wt%  
46 and then increase of the concentration of the precursor in  
47 the initial solution to 5 wt%. Furthermore, the character of  
48 the dependencies is changed. For 0.5 wt% of THEOS, the  
49 curves 3 and 4 are almost merged. Their slope is decreased  
50 in comparison with curves 1 and 2. The  $G'$  and  $G''$  moduli  
51 scale with frequency with an exponent of 1/2.

52 With the silica precursor concentration increases to  
53 5 wt%, a curve 5 corresponding to the storage modulus  
54 is shifted up more than that relative to the loss modulus  
55 (curve 6), thus being above the other. In addition, the slope  
56 is further decreased. When  $G'$  exceeds  $G''$  in the whole

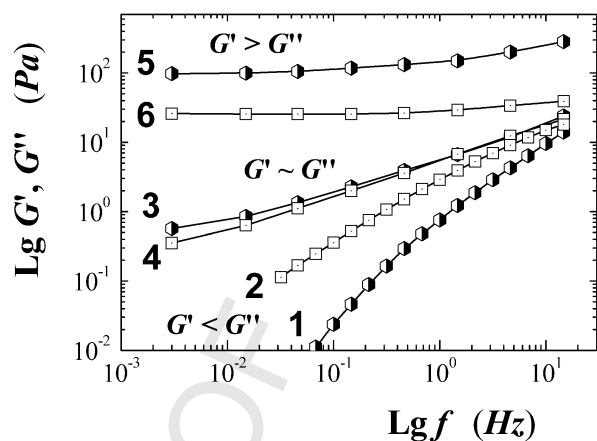


Fig. 1. The storage modulus  $G'$  (1, 3, 5) and loss modulus  $G''$  (2, 4, 6) vs the oscillation frequency  $f$ . All the measured systems contained 1.5 wt% of cat-HEC. Hydrogels were prepared by introducing 0.5 (3, 4) and 5 wt% (5, 6) of THEOS into a polysaccharide solution.

frequency range available for the measurement, this is characteristic of solid or solidlike (soft) materials [32–34]. In the case of polymer solutions, this means that the macromolecules are cross-linked through covalent bonding [32,34].

Thus, it follows from the data presented in Fig. 1 that the silica introduced into the cat-HEC solution resulted in a transition from a liquid to a solidlike state. The set of curves usually serves to provide support for the fact of sol–gel transition in the system. As this takes place, there is a sol–gel point at which  $G' \approx G''$  [34–36]. This case is represented by curves 3 and 4 in Fig. 1, which almost exactly match in the whole frequency range. Therefore, we may conclude that the silica generated in situ by the sol–gel processing provides gelation of the cat-HEC solutions. This happens when only 0.5 wt% of THEOS is introduced. For other polysaccharides, the concentrations  $C_{s-g}$  of silica precursor at which the transition into the gel state takes place are given in Table 1. As seen, there is a notable difference in the THEOS concentrations at which gelation is reached. This fact argues for a dependence of the sol–gel transition on the nature of the polysaccharide.

It is significant that the silica precursor in itself did not have the capability to gel an aqueous solution under the same conditions if a polysaccharide was not introduced. To perform the sol–gel processing, it was necessary, as usual (see Refs. [16,17]), to add a catalyst (acid or alkaline). In this case, a monolithic gelled material was formed when no less than 20 wt% of THEOS was mixed with water. At a smaller precursor concentration phase separation or silica precipitation was observed. Monolithic hydrogels with decreased amounts of silica were fabricated without addition of the catalyst on the basis of polysaccharide-containing solutions.

The mechanical properties of hydrogels depended on the polysaccharide and precursor concentrations added into the initial solution. Dependences of the zero-shear viscosity and plateau modulus on the THEOS concentration are given in Figs. 2A and 2B, respectively, by the examples of cat-HEC

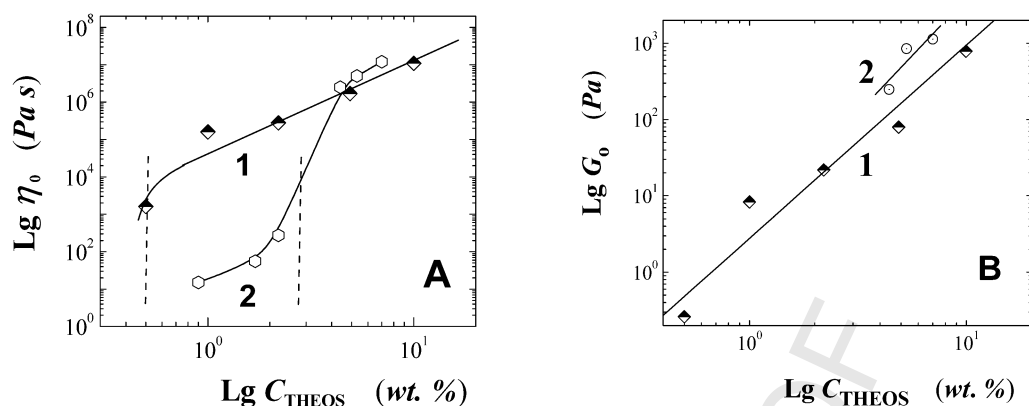


Fig. 2. The zero-shear viscosity (A) and plateau modulus (B) vs the concentration of THEOS in the initial solution containing 1.5 wt% of cat-HEC (1) or sodium hyaluronate (2).

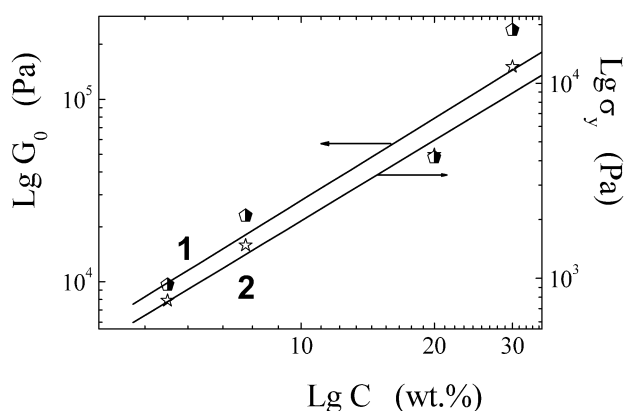


Fig. 3. The plateau modulus (1) and yield stress (2) vs the concentration of THEOS in the initial solution containing 5 wt% of  $\alpha$ -cyclodextrin.

(curve 1) and sodium hyaluronate (curve 2). The sol-gel points are marked by dashed vertical lines to show how they differ in the considered systems (Fig. 2A). A transition into the gelled state is accompanied by a sharp increase of the solution viscosity. It is well seen in the case of the hyaluronate-containing system (curve 2, Fig. 2A). Further addition of the silica precursor resulted in an increase of the mechanical strength of hydrogels. This is obvious from the concentration dependence of the plateau modulus (Fig. 2B). A power law exponent for the cat-HEC-containing system, found from the best linear fit to the data (curve 1), is 2.5. This means that the increase of silica concentration strengthens the polysaccharide hydrogel effectively.

Fig. 3 presents the plateau modulus and yield stress against THEOS concentration for hydrogels containing 5 wt% of  $\alpha$ -cyclodextrin. The gelation in the presence of cyclodextrins differed from that of previously considered cat-HEC and sodium hyaluronate (Fig. 2). The sol-gel processing, when small precursor amounts were added, was accompanied by precipitation of reaction products. A homogeneous hydrogel was formed at a rather large critical concentration of THEOS (ca. 4 wt%). The following increases of precursor concentration, as obvious from Fig. 3, resulted in growth of its mechanical strength. This is in line

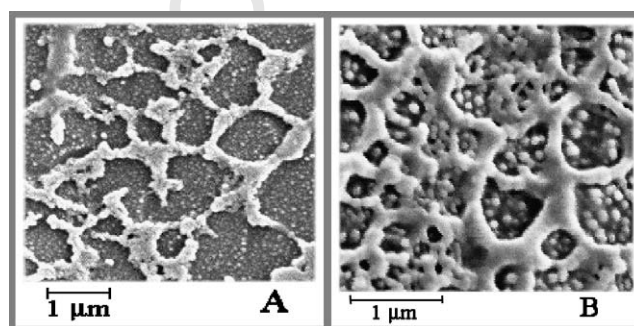


Fig. 4. SEM micrographs of hydrogels synthesized by introducing 10 wt% THEOS into a solution with 0.5 wt% sodium hyaluronate (A) or 1.5 wt% cat-HEC (B).

with results obtained with cat-HEC and sodium hyaluronate, but there is also a difference in the slope of straight line fitted to the experimental data. A power law exponent for the concentration dependence of the plateau modulus was found to be equal to 1.5. This value is notably lower than the power law exponent for cat-HEC, which comes out to 2.5. The variation of scaling behavior with a change of polysaccharide in the hybrid nanocomposites was also mentioned in [19], but the reason for it is not understood at present. **CAUSE**

The reason for the gelation of polysaccharide solutions after the silica generation in situ by sol-gel processing becomes evident if one examines hydrogel images taken by the scanning electron microscope. They are presented in Fig. 4. There are pictures of hydrogels prepared by the mixing of 10 wt% of THEOS with 0.5 wt% of sodium hyaluronate (Fig. 4A) or 1.5 wt% of cat-HEC (Fig. 4B). As seen, their main morphological elements are a network consisting of cross-linked fibrils and un-cross-linked spherical particles filling the mesh space. Both the former and the latter are made up of silica. The fibrous structures represent macromolecules that initially form a three-dimensional network in the solution bulk. Then they were covered, as shown by the AFM in Ref. [37], by a shell of precipitated silica. The spherical particles could be separated macromolecules or silica sol that was fabricated in the initial stages of silica polycondensation [22,23].

1 It should be pointed out that the suggested mechanism  
2 is valid for all the linear and branched polysaccharides (Ta-  
3 ble 1). The cyclodextrins do not form a three-dimensional  
4 network from entangled macromolecules. They are inte-  
5 grated into the structure generated by silica. This was shown,  
6 for example, in [25,26]. However, the cyclodextrins had a  
7 strong catalytic effect on the sol–gel processing, initiating  
8 them even when were taken in trace amounts.

9 The foregoing results demonstrated that the sol–gel tech-  
10 nology can be used efficiently where it is necessary to gel  
11 solutions of nongelable polysaccharides. It is worth men-  
12 tioning that this happens at small precursor concentrations  
13 reaching even tenths of a percent in solutions (Fig. 2, Ta-  
14 ble 1). The commonly used precursors, TMOS and TEOS,  
15 are inapplicable of doing it in such amounts. Furthermore,  
16 they are not compatible enough with biopolymers [31,38].  
17 The gelation of polysaccharide solutions demonstrated in  
18 this work was possible owing to advantages of THEOS over  
19 the common precursors. It is completely water-soluble [16,  
20 17] and its sol–gel processes are catalyzed by polysaccha-  
21 rides that made it possible not to add an organic solvent  
22 or/and a catalyst [18,19]. What is also important is that the  
23 produced polysaccharide–silica hydrogels are biocompatible  
24 materials because of the compatibility of both organic and  
25 inorganic components with living systems. This holds great  
26 promise for their various applications. It will suffice to men-  
27 tion that the gelation of sodium hyaluronate and chitosan  
28 solutions is a real challenge [6,10,39,40]. As demonstrated  
29 here, it can be solved using a nanotechnological approach.

### 31 Acknowledgments

32 The authors are indebted to Dr. C. Abetz and I. Otto  
33 (Bayreuth University) for the SEM micrography of hydro-  
34 gels as well as to Dr. T. Yanaki and Prof. Dr. T.N. Zvyagintseva  
35 for the presented polysaccharides.

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