

Formation features and AFM studies of the silicon-chitosan-containing glycerohydrogel surface

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Abstract. Hybrid glycerohydrogels based on silicon tetraglycerolate and chitosan were produced by means of one-pot sol-gel synthesis. The influence of the component composition (the concentration of the template and precursor), the low-molecular-weight accelerator (NaCl), and the conditions of the process (pH, temperature) on the gelling duration was estimated. The glycerohydrogel surface microrelief was visualized by AFM in the semi-contact mode and the standard roughness parameters were calculated. The silicon-chitosan-containing glycerohydrogels were established to have a complex surface relief. Their solid sol-gel matrix is characterized by a uniform distribution of elements of the inorganic phase and a developed microporous structure with amorphous-crystalline ordering. The scale of the surface irregularities, as well as their structural and morphological features and the crystallinity degree of the solid sol-gel matrix, are determined by the template/precursor weight ratio and the molecular weight of the polymer.

1. Introduction

Inorganic/organic hybrid hydrogels are promising materials for application in medicine and pharmacology [1]. Such hydrogels are prepared by introducing an inorganic phase precursor (commonly silicon alkoxy compounds $\text{Si}(\text{OR})_4$) into a polymer solution, resulting in the sol-gel synthesis of an inorganic network of siloxane bonds on an organic template matrix [2–4]. The possibility of one-pot synthesis of homogeneous monolithic hydrogels is an essential merit of sol-gel technology [5, 6].

The nature of the precursor and template, which determines the composition and structure of the hydrogel system [4, 6–8] and has a significant impact on the spectrum of its biologically useful properties [3, 4, 9–12], is the most important factor in obtaining hybrid hydrogels. E.g., the use of an ion-crosslinked biologically active chitosan-containing matrix as the organic phase allows obtaining biocompatible hydrogels with high mucoadhesive, bacteriostatic, and immunocorrecting properties [9, 10]. Using Si tetraglycerolates as the inorganic network precursor leads to the formation of silicon-chitosan-containing glycerohydrogels with haemostatic and wound-healing activity [3, 4, 6]. In this case, the precursor hydrolysis results in a spatial network of $\equiv\text{Si}-\text{O}-\text{Si}\equiv$ bonds, and the chitosan macromolecules serve as a template during gelation [4, 6–8]. Besides, the inorganic phase formation proceeds non-catalytically under mild conditions (close to the physiological ones) to form a non-toxic by-product (glycerol), being a solvent of the resulting silicon polyolates; all this leads to the production of glycerohydrogels [11, 12].

In addition, chitosan aerogels, cryogels, and xerogels, formed from the corresponding hybrid hydrogels, are characterized by high porosity, large specific surface, satisfactory strength, and kinetic



stability (not subject to syneresis) [4, 5]. This makes it possible to use chitosan-containing solid sol-gel matrices as bioadhesive tissue engineering designs (bioceramics, bio-glass) for the restoration of bone and cartilaginous tissues [13]. The presence of macro- and mesopores can be used to load drugs and growth factors; it also promotes faster tissue germination and filling of large-volume defects [14]. The dehydration method (sublimation or supercritical drying, freezing-thawing or extraction), as well as the formation conditions of the initial hydrogels, are the determining factors in the formation of a predetermined structure of the solid sol-gel matrix.

The surface roughness scale of hydrogel systems is known to determine the adhesion and proliferation of cells, and to be responsible for their dermoadhesive and mucoadhesive properties [15]. The solid sol-gel matrix surface topography plays an important role at the first stages of reparative osteogenesis. At present, atomic force microscopy (AFM) is the most promising direct method for analyzing the surface structure. This technique allows studying the surface geometry and local properties of a hydrogel sample with high spatial resolution and contrast [16, 17]. In addition, AFM studies in a semi-contact mode allow minimizing mechanical damage to the soft hydrogel surface. The subsequent digital data processing allows an in-depth analysis of the sample microrelief. To evaluate structural-morphological features of the solid sol-gel matrix, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray analysis are considered as the most informative ones [18, 19].

The purpose of this work was to prepare silicon-chitosan-containing glycerohydrogels by sol-gel technology and to explore structural-morphological features of the surface of this glycerohydrogel material and its solid phase using AFM, SEM, EDX, and X-ray diffraction analysis. A silicon tetraglycerolate solution in a two-molar glycerol excess ($\text{Si}(\text{OGly})_4 \cdot 2 \text{ GlyOH}$) and an ion-crosslinked hydrogels of chitosan glycolate were used as the precursor and template, respectively, for the preparation of hybrid glycerohydrogels. Preparing the ion-crosslinked chitosan matrix is based on the salt formation process occurring when chitosan is dissolved in an aqueous solution of glycolic acid [8]. The choice of this acid is due to the fact that it belongs to the class of pharmacopoeial substances (UNII: 0WT12SX38S), exhibits antioxidant properties, and promotes cell regeneration and moisture retention in tissues [20]. Due to these biologically useful properties, it is used to produce pharmaceutical, dermatological, and cosmetic preparations with keratolytic and rejuvenating properties for the treatment of acne, scars, and depigmentation [21].

2. Methodology

2.1. Materials

The following reagents were used: powders of chitosan with a viscosity-average molecular weight 38 (LCS) and 200 kDa (HCS), a deacetylation degree of 70 and 82 mol.%, respectively (Bioprogress Ltd., RF); tetraethoxysilane $\text{Si}(\text{OEt})_4$ (Ekos-1 Ltd., RF); glycerol (GlyOH) (Vekton Ltd., RF); glycolic acid 70% $\text{C}_2\text{H}_4\text{O}_3$ (GIA) (Sigma-Aldrich, USA); NaCl and NaOH (NPO EKROS Ltd., RF); distilled water. All low-molecular-weight chemicals were of analytical grade or higher and used without further purification; freshly prepared solutions were always used in all experiments.

2.2. Synthesis of organically modified silicon

Silicon tetraglycerolates were synthesized by transesterification of tetraethoxysilane in a polyol excess without a catalyst according to Larchenco et al. [4]:



The first step was esterification between tetraethoxysilane and glycerol within 70-80°C under constant stirring until the phase interface disappeared. At the second step, the formed free EtOH was distilled off from the reaction mixture at 80°C under atmospheric pressure. The completion of this stage was controlled by cooling to 73°C and by the volume of the EtOH distilled off as an azeotrope (~75% of the theoretical value). At the third step, EtOH was removed from the system at 140°C under

15 mm Hg for 3 h. The reaction completeness and the purity of the inorganic phase precursor were monitored using IR and ^1H NMR spectroscopy, by no signals of $>\text{C}=\text{O}$ and $>\text{C}=\text{C}<$ within $1705\text{--}1685\text{ cm}^{-1}$ and those of $-\text{O}-\text{C}_2\text{H}_5$ within $1.1\text{--}2.3\text{ ppm}$, respectively, and also by refractometry [22].

2.3. Preparation of chitosan solution

Chitosan (CS) solutions with a polymer concentration $C_{\text{CS}} = 4\%$ (w/v) in aqueous glycolic acid (GIA) with a concentration of $C_{\text{GIA}} = 1.5\%$ (v/v) were used. The choice of such polymer concentration was due to the fact that chitosan accelerated gelation in weakly acidic media, when $C_{\text{CS}} > 1\%$ (w/v) [6–8]. The solutions were prepared by dissolving polymer powder in an aqueous GIA solution at the ambient temperature without light for 24 h. As a result, chitosan glycolate (CS-GIA) was formed. The pH of the system was adjusted by adding a 0.4 M aqueous NaOH solution.

2.4. Sol-gel synthesis of silicon-chitosan-containing glycerohydrogels

To synthesize silicon-chitosan-containing glycerohydrogels, chitosan and $\text{Si}(\text{OGly})_4 \cdot 2\text{ GlyOH}$ solutions were used in several weight ratios ($5:1 \div 1:13$) with/without a low-molecular-weight accelerator (NaCl powder, 1 wt.%). The component concentrations were controlled gravimetrically on Ohaus Adventurer AR 1530 scales (the accuracy of weighing $\pm 0.002\text{ g}$) and were expressed in wt.%. The mixed solutions were stirred carefully until homogeneity (1–2 min) and kept at 4 ± 0.5 , 20 ± 2 , or $37 \pm 0.5^\circ\text{C}$ under atmospheric pressure for sol-gel synthesis. The gel point was fixed by the loss-flow time, using “the tube inverting method”. The gel point corresponded to the instant of time, after which the system did not flow within 40 s after the tube had been turned over at a given temperature. The concentrations of chitosan and silicon in the obtained glycerohydrogels varied in the range of $C_{\text{CS}} = 0.7\text{--}3.7\text{ wt.}\%$ and $C_{\text{Si}} = 0.4\text{--}2.4\text{ wt.}\%$, respectively. The template/precursor weight ratio ($C_{\text{CS}}/C_{\text{Si}}$) was used to express the component composition of each mixture.

2.5. Solid phase preparation

The solid phase (xerogel) was prepared by exhaustive cold extraction combined with cryotreatment and re-extraction. Samples were placed into EtOH (95.6%) for 30 days. The ethanol has been chosen, because it is a non-solvent (precipitant) for chitosan and silicon glycerohydrogel, but is a solvent for water, glycolic acid, and glycerol. The precipitant was replaced every 10 days. Then, the samples were cryofrozen in a refrigerator (Sanyo mdF-U3286S) at -85°C for 24 h and were re-kept in the precipitator (alcohol/acetone) for 3 days followed by drying in a desiccator (CaCl_2) during 24 h (Fig. 1).

2.6. Methods

The physicochemical parameters (pH and refractive index n_D^{25}) of the mixed solutions were measured on a Mettler Toledo Five Easy FE20 pH-meter and a Mettler Toledo RM40 refractometer (Germany).

IR spectra were recorded on a Nicolet IR-6700 spectrometer, FT-IR (USA) with a resolution of 4 cm^{-1} in the range of $4000 \div 5000\text{ cm}^{-1}$, using a mean of 32 scans.

The ^1H NMR spectra of the synthesized silicon tetraglycerols were recorded on a VARIAN-400 (USA) spectrophotometer within a frequency range of $5.4\text{--}2.0\text{ ppm}$, 400 MHz. A solution of silicon tetraglycerolate (20 μl) in D_2O (480 μl) was used.

X-ray diffraction analysis was performed on a DRON-3 diffractometer with $\text{Cu-K}\alpha$ emission at $U = 22\text{ kV}$ and $J_a = 20\text{ mA}$. The crystallinity degree χ (%) was estimated by the method from Ref. [23] as the ratio of the total scattering from crystallites to the total scattering from both amorphous and crystalline regions:

$$\chi = [\int_0^\infty s^2 I_c(s) ds / \int_0^\infty s^2 I(s) ds] \cdot 100\%,$$

where $s = (2 \sin\Theta)/\lambda$, 2Θ is the diffraction angle (deg), λ is the X-ray wavelength equal to 1.54 \AA ; $I(s)$ and $I_c(s)$ are the intensities of coherent X-ray scattering from the entire sample and that from its crystalline region, respectively (rel. units).

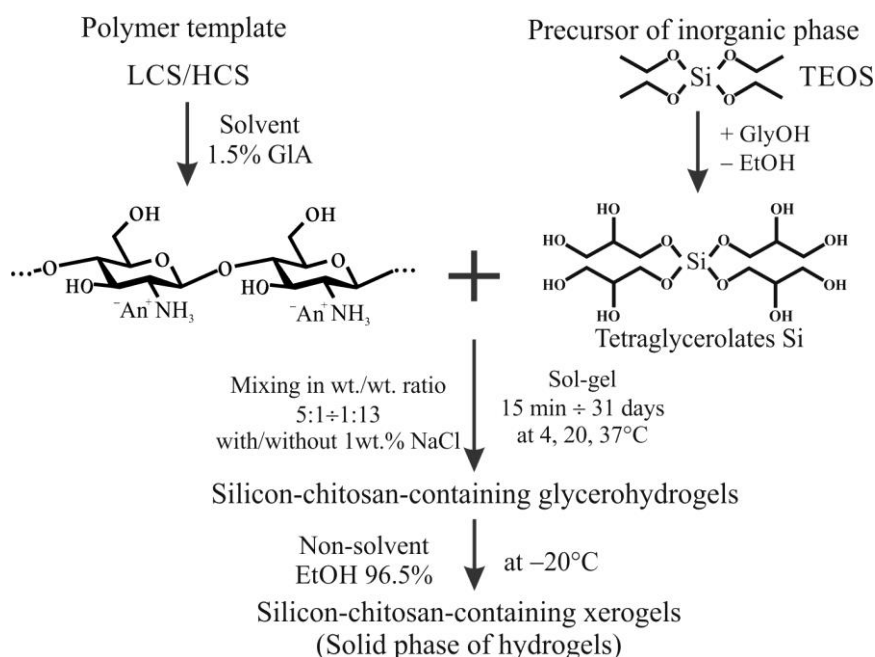


Figure 1. A scheme of the preparation of silicon-chitosan-containing glycerohydrogels and xerogels.

A graphical method was used to calculate χ (%). The X-ray diffractograms were digitized, the boundary between the amorphous halo and crystal peaks (the Paint editor) was drawn, and the areas of the resulting curvilinear figures were found (AreaS software).

The surface morphology and the Si content (C_{Si}^*) in the surface layer of xerogel samples were evaluated by SEM and EDX on a MIRA/LMU scanning microscope (Tescan, Czech Republic), equipped with a INCA Energy 350 system (Tescan, Czech Republic) at a voltage of 8 kV and a conductive current of 60 pA. A 5-nm-thick golden layer was sprayed onto each sample with a magnetron sputtering installation K450X carbon coater (Germany) at a spraying current of 20 mA and a spraying duration of 1 min. The resolution was 3 nm, the INCA Energy detector sensitivity was 133 eV/10 mm². All studies were carried out in vacuum (10⁻² Pa).

For image acquisition in a semi-contact mode, NSG10 probes (NT-MDT-SI Ltd., Russian Federation) with a resonance frequency around 220 kHz, a force constant 3–38 N×m⁻¹ and a tip curvature less than 10 nm were used. AFM images of the surface of glycerohydrogels were processed by the *Gwyddion* 2.48 software [24] for the background flattening and for smoothing of three pixel length median filter to eliminate single-pixel noise emissions. Two of the standard roughness parameters: the mean roughness Ra and the root-mean-square roughness Rq were calculated.

3. Results and discussion

3.1. Study of the gelation time of silicon-chitosan-containing glycerohydrogels

To evaluate the effect of the component composition and the sol-gel synthesis conditions on the gelation time of silicon-chitosan-containing glycerohydrogels, mixed compositions with several template/precursor weight ratios (C_{CS}/C_{Si}) were prepared and held at three temperatures for the sol-gel process: the standard storage temperatures for pharmaceutical preparations (4 ± 0.5 and $20 \pm 2^\circ\text{C}$) and the physiological temperature ($37 \pm 0.5^\circ\text{C}$). In addition, the pH of the mixed formulations was varied by adding NaOH to the CS-GIA stock solution. As the pH increased, the amount of CS deprotonated amino groups increased without precipitation of the polymer. To reduce the gelation time, a low-molecular-weight accelerator (NaCl) with the least toxic effect was chosen from the series of lyotropic anions [3]. In all cases, the formation of monolithic shape-stable transparent light-beige systems was completed within each sol-gel reaction (Fig. 2).

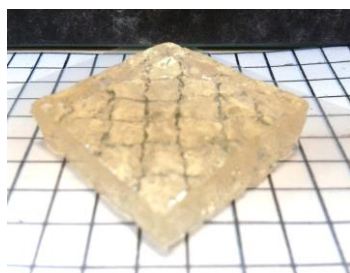


Figure 2. A sample of obtained silicon-chitosan-containing glycerohydrogel.

Figure 3 shows the dependence of the gelation time of the mixed compositions based on $\text{Si}(\text{OGly})_4$ solutions in glycerol, low- and high-molecular-weight CS in GlA, on the template/precursor weight ratio for several pH values and temperatures. It can be seen that for all compositions in the whole studied concentration range of the polymer template and precursor, there is a general tendency of increasing the gelation time with an increase in the $C_{\text{CS}}/C_{\text{Si}}$ ratio. Increasing the temperature and pH of the medium reduces the gelation time by ~ 1.5 –12 times on average.

The electrolyte salt addition (NaCl) in an amount of 1 wt% further increased pH and reduced the gelation time of the mixed compositions (up to ~ 30 times) as compared to the initial composition with no accelerator. At the same time, the molecular weight of the polymeric template had no significant effect on time of reaching the gel point.

For more detailed study of the pH effect, a system based on LCS and $\text{Si}(\text{OGly})_4 \cdot 2 \text{ GlyOH}$ with $C_{\text{CS}} = 2.67$ wt.% and $C_{\text{Si}} = 1.62$ wt.% was chosen, which gelation time in an acidic medium did not exceed 4 h. As expected, gelation time of this composition decreased monotonically with increasing pH (Table 1). E.g., in a strongly acidic medium ($\text{pH} < 4.2$), gelation took several hours, while that in a slightly acidic medium ($\text{pH} 4.3$ – 5.0) took 30 min to 1 h. For $\text{pH} 5.5$ – 6.0 (close to neutral), gelation occurred within few minutes. Deceleration of the sol-gel transition in the lower pH range could be explained by the formation of a stable cationic complex of silicon glycerolate with the acid, where the silicon atom is in a coordinated saturated state [4, 6].

To evaluate the influence of the polymer template concentration on the gelling process, systems were chosen based on LCS and HCS with a constant precursor concentration ($C_{\text{Si}} = 1.62$ wt.%) and the same pH (4.5). Gelation was carried out at 37°C . As can be seen from Figure 4, both compositions are characterized by a reduced gelation time with increasing of the chitosan content. However, this effect is more pronounced for the HCS-based system than for the LCS-based one.

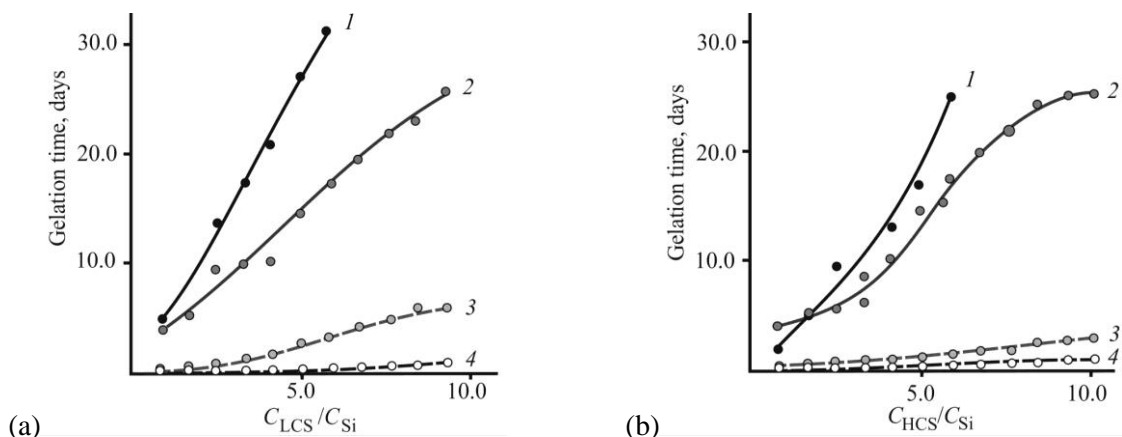
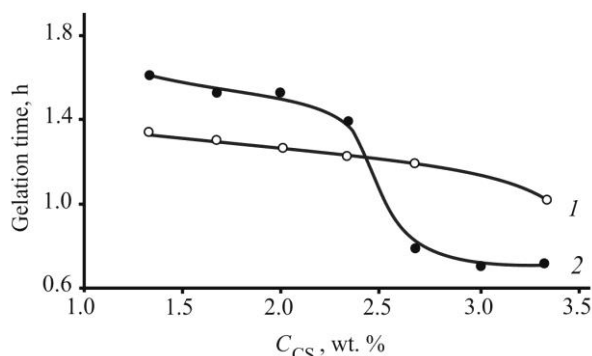


Figure 3. Dependence of the gelation time on $C_{\text{CS}}/C_{\text{Si}}$ for the systems (a) LCS-GlA and (b) HCS-GlA + $\text{Si}(\text{OGly})_4 \cdot 2 \text{ GlyOH}$ at several pHs: (a) 2.3 (1), 3.8 (2, 3), 4.3 (4) and (b) 3.3 (1), 3.8 (2, 3), 4.2 (4); the incubation temperature 4°C (1, 2), 20°C (3) and 37°C (4); without (1–3) and with addition of 1 wt.% NaCl (4).

Table 1. Dependence of the gelation time on pH for the system with $C_{LCS} = 2.67$ wt.% and $C_{Si} = 1.62$ wt.% at 37°C .

pH	Gelation time, h
3.8	3.6
4.0	2.3
4.2	2.0
4.7	0.8
5.0	0.5
5.5	0.3
5.7	0.2
6.0	0.1

**Figure 4.** Dependence of the gelation time on C_{CS} for the system based on LCS (1), HCS (2) + $\text{Si}(\text{OGly})_4 \cdot 2 \text{GlyOH}$ at $C_{Si} = 1.62$ wt.%, 37°C , and $\text{pH} = 4.5$.

The dependence of the gelation time on the chitosan concentration (Fig. 4) correlates with the pH dependence (Table 1). The obtained dependence (Fig. 4) confirms once more the assumption from Ref. [6] that numerous intermolecular bonds are formed in a weakly acidic or neutral medium, including hydrogen bonding between the free (deprotonated) NH_2 groups of the template macromolecules and the Si-OH groups formed as a result of the precursor hydrolysis. The additional nucleation centers thus formed have an accelerating effect on the gelation. The template/precursor weight ratio in the mixture composition, which determines the hybrid glycerohydrogel morphology, is also important in this process.

Based on the obtained results, the following samples compositions were selected for the analysis of their structural-morphological features: with template/precursor weight ratios $C_{CS} \gg C_{Si}$, $C_{CS} \approx C_{Si}$, and $C_{CS} \ll C_{Si}$. The concentrations of the main components and the symbolic designations for the samples studied are given in Table 2.

3.2. AFM studies of the surface microrelief of glycerohydrogels

Topography visualization and a quantitative assessment of the surface microrelief of the obtained hybrid glycerohydrogels were performed by AFM. Analysis of the AFM images of surface areas (the scanning area size of 2×2 and $10 \times 10 \mu\text{m}^2$) showed that all samples were characterized by a complex surface relief (Figs. 5 and 6). Two types of structural irregularities were observed: protrusions and depressions (of spherical or irregular shape).

The alternation of these protrusions and valleys was fairly uniform. Sections were drawn on the AFM images, along which profiles of the surface roughness were plotted. The sizes of these irregularities (their height, depth, pitch, and diameter) and two roughness parameters (the arithmetic mean Ra and the mean-square roughness Rq) were estimated.

Table 2. Composition of the silicon-chitosan-containing hydrogel samples.

Sample	Chitosan	Composition, wt. %		Component weight ratio C_{CS}/C_{Si}	Designation
		C_{CS}	C_{Si}		
1	LCS	3.3	0.8	4.1	LCS-4.1
2		2.0	2.4	0.8	LCS-0.8
3		0.7	4.1	0.2	LCS-0.2
4	HCS	3.3	0.8	4.1	HCS-4.1
5		2.0	2.4	0.8	HCS-0.8
6		0.7	4.1	0.2	HCS-0.2

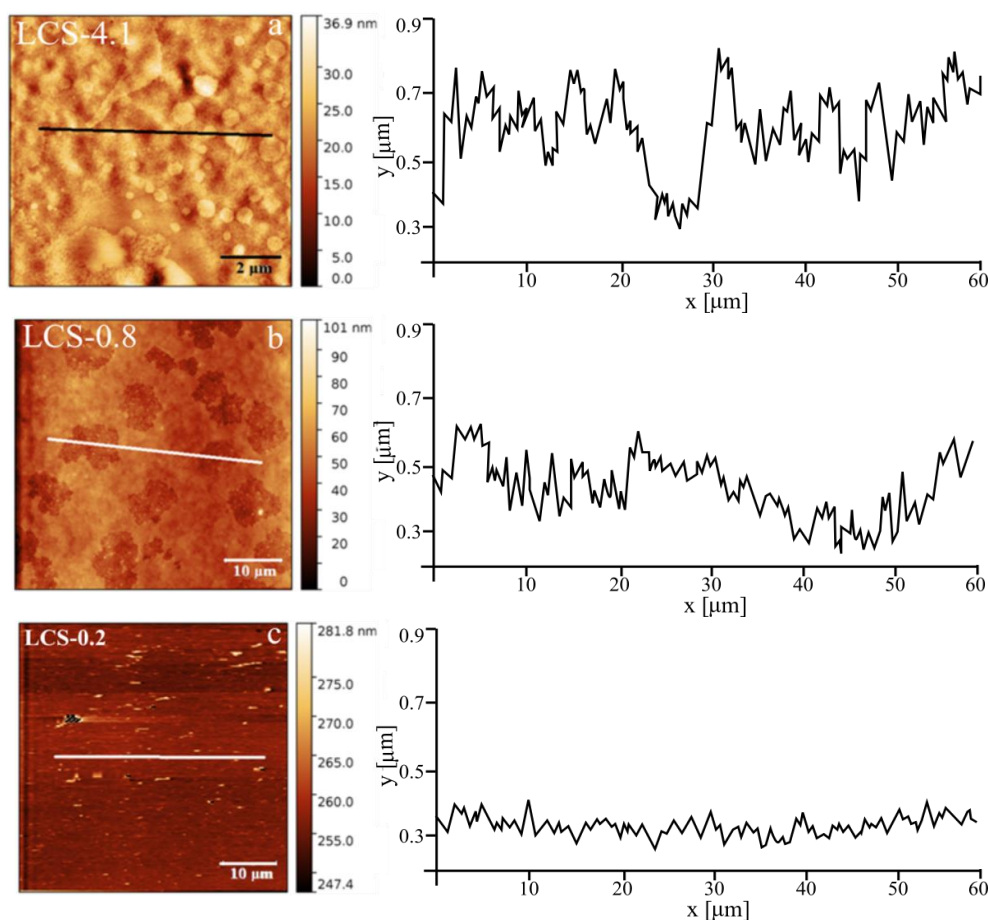


Figure 5. AFM image and roughness profile of the surface of LCS-based silicon-chitosan-containing glycerohydrogels. The designation of the samples is indicated in Table 2.

Figure 5 shows the AFM image and surface roughness profile of the obtained hybrid LCS-based glycerohydrogels. These samples are characterized by a granular relief with the predominance of protrusions of ~40–250 nm in height and a vertical pitch of ~75–500 nm. Statistical analysis showed that the mean arithmetic roughness of the surface varied within the range of $Ra = 4\text{--}17$ nm, and the mean-square roughness varied within the range of $Rq = 5\text{--}22$ nm (Table 3). The graininess and roughness scale is determined by the C_{LCS}/C_{Si} weight ratio: the surface irregularities naturally increase with the LCS content in the samples. The samples of our hybrid HCS-based and LCS-based glycerohydrogels are characterized by a granular relief, with the predominance of depressions of ~30–70 nm in depth and ~15–3 μm in diameter (Fig. 6). With decreasing the chitosan content in the sample, the diameter of the depressions decreased, and their surface concentration (the number of surface cavities per unit area) increased. This was the most characteristic for HCS-0.2. The roughness of these samples was also determined by the HCS content in the glycerohydrogel, but there was no clear pattern of the effect of the C_{HCS}/C_{Si} weight ratio on the micro-relief profile of the hydrogel surface. The mean arithmetic surface roughness of HCS-based samples was $Ra = 10\text{--}32$ nm, the mean-square roughness was $Rq = 15\text{--}40$ nm (Table 3).

3.3. Study of the solid phase (xerogels) of hybrid glycerohydrogels by EDX, SEM, and XRD

A quantitative analysis of the inorganic phase composition was carried out and structural-morphological features of the solid samples (xerogels) prepared from the corresponding glycerohydrogels by exhaustive cold extraction in combination with cryogenic processing and subsequent freeze drying were evaluated.

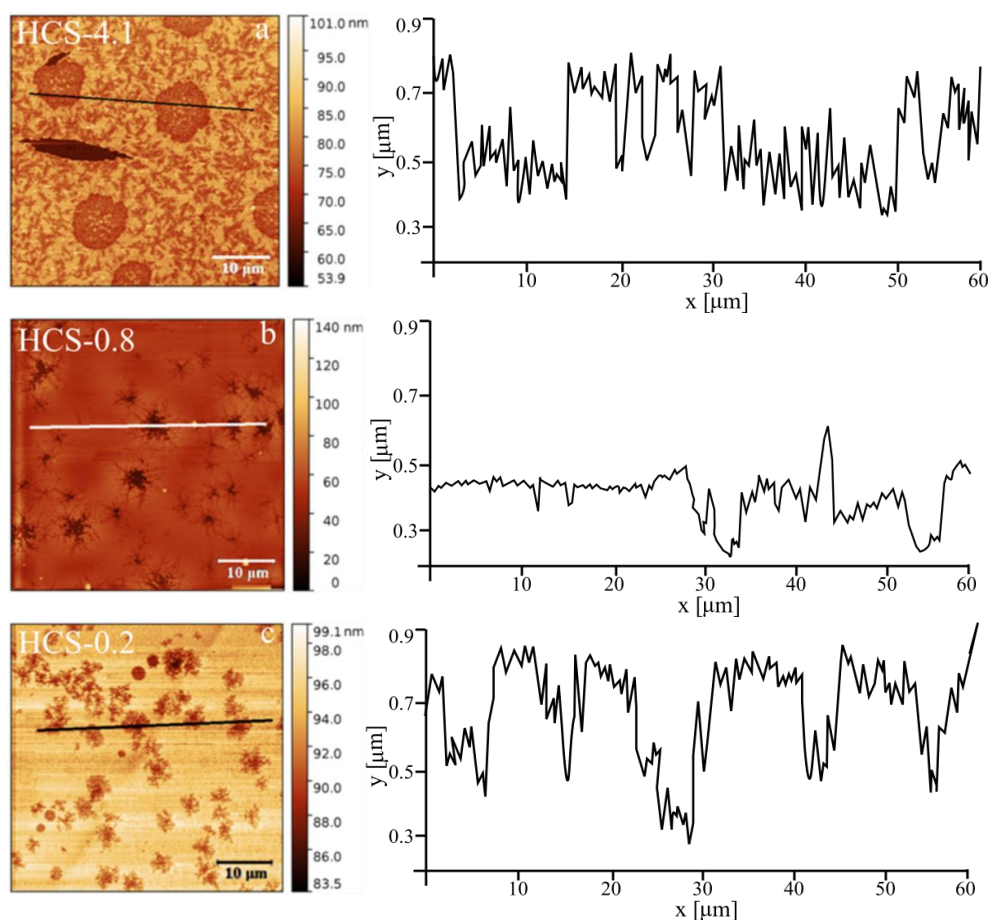


Figure 6. AFM image and roughness profile of the surface of HCS-based silicon-chitosan-containing glycerohydrogels. The designation of the samples as in Table 2.

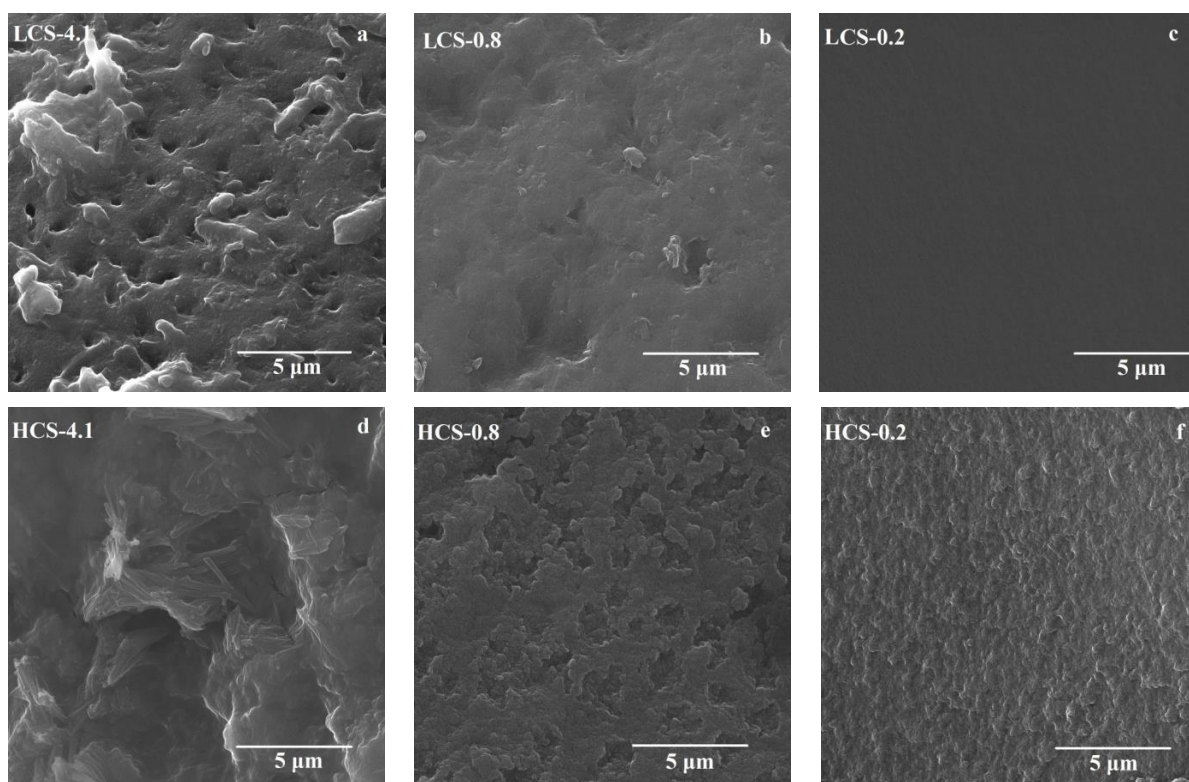
The results of our quantitative estimation of the silicon content (C_{Si}^*) in the surface layer are presented in Table 3 (HCS-based xerogels). Attention is drawn to the correlation of the C_{Si}^* values in the solid samples with the calculated C_{Si} values in the corresponding glycerohydrogels (Table 2), which indicates a uniform distribution of the inorganic phase over the synthesized samples.

The SEM images show that the solid phase of the samples is represented by a developed microporous structure (Fig. 7). The samples with the highest chitosan contents (a, d) are characterized by a more loose structure with the largest number of aggregates formed from the surface, relatively uniform in the size of pores and channels. The greatest amount of these inhomogeneities was observed for the xerogel obtained from the glycerohydrogel based on LCS-4.1. As the polymer template content decreased, both in the system on the basis of LCS (a-c) and HCS (d-f), a tendency of leveling structural heterogeneities was observed. For the xerogel obtained from the LCS-0.2 sample with the lowest template content, an almost smooth surface (c) was observed. This difference, as well as smoothing of the relief with a decreased CS content, can be due to changes in the structure (e.g., compression) of the sample during the liquid phase removal. The lower the content of the structure-forming component (the template) in the glycerohydrogel sample, the higher the stresses arising during the xerogel preparation and the more likely the process of collapsing the initially highly porous structure.

The X-ray diffractograms of the xerogels are typical for amorphous crystalline polymers with a low crystallinity degree (Fig. 8).

Table 3. Physicochemical characteristics of the silicon-chitosan-containing glycerohydrogels and xerogels.

Sample	Hydrogels		Xerogels			
	R_a , nm	R_q , nm	2Θ , deg	d , Å	χ , %	C_{Si}^* , wt. %
	AFM-data		RSA-data		EDX-data	
LCS-4.1	17	22	22.8	3.90	25.3	—
LCS-0.8	9	15	23.0	3.87	10.6	—
LCS-0.2	4	5	24.6	3.48	12.5	—
HCS-4.1	32	40	27.0	3.30	18.7	8.39
HCS-0.8	10	15	23.0	3.87	14.2	28.08
HCS-0.2	24	32	23.0	3.87	8.4	39.23

**Figure 7.** SEM images of the solid phase (xerogels) of the obtained silicon-chitosan-containing glycerohydrogels based on LCS (a-c) and HCS (d-f). The xerogel sample designation corresponds to that of the glycerogels, which they were obtained from (Table 2).

Against an amorphous halo background, reflections appear, characteristic of the crystalline structure of polymorphic chitosan at $2\Theta = 22.8\text{--}24.6$ deg (LCS) and $2\Theta = 23.0\text{--}27.0$ deg (HCS). The data on the interplanar spacings (d , Å) in the crystal lattices of the samples under study are close to each other (Table 3) and agree with the known literature data for chitosan films [25, 26].

The calculated crystallinity degree (χ) of solid samples decreased with increasing of the inorganic phase fraction (Table 3). For the LCS-based and HCS-based xerogels, the crystallinity degree was 25.3–10.6% and 18.7–8.4%, respectively. The lower crystallinity degree of the HCS-based xerogels could be explained by the more limited mobility of segments in the macromolecules of high-molecular-weight chitosan as compared to that with a lower molecular weight, which makes it difficult to form full-length long-range order.

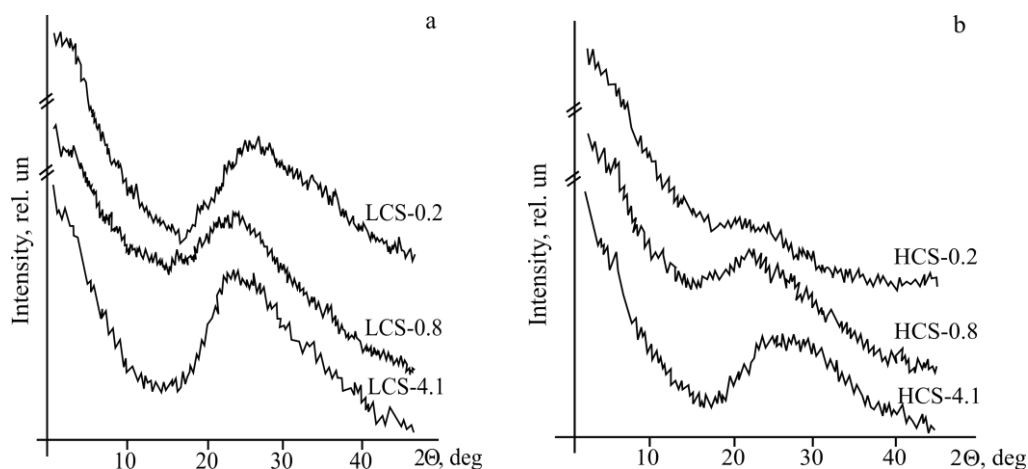


Figure 8. X-ray diffraction patterns of the xerogels of our silicon-chitosan-containing glycerohydrogels based on (a) LCS and (b) HCS. The xerogel sample designation corresponds to that of the glycerogels, which they were obtained from (Table 2).

4. Conclusion

Silicon-chitosan-containing glycerohydrogels based on HCS, LCS, and $\text{Si}(\text{OGly})_4$ were prepared by sol-gel synthesis. The influence of the composition and the conditions of the process on the gelation time was evaluated. It was found that at pH values close to neutral, the gelation in the systems under study could proceed during 5–15 min.

Structural-morphological features of the solid phase of glycerohydrogels were visualized and evaluated. When $C_{\text{CS}} \gg C_{\text{Si}}$, the xerogel specimens were found to be characterized by a more loose structure, higher values of their roughness parameters, and a higher crystallinity degree. When $C_{\text{CS}} \ll C_{\text{Si}}$, a tendency was observed to smoothing out the heterogeneities and amorphization of the structure. Thus, variations of the composition of the template-precursor mixtures and the synthesis conditions allow obtaining silicon-chitosan-containing materials with the desired properties.

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References

- [1] Samal S, Dash M, Van Vlierberghe S and Kaplan D 2012 *Chem. Soc. Rev.* **41** 7147-94
- [2] Shchipunov Y A, Karpenko T Y and Krekoten A V 2005 *Compos. Interfac.* **11** 587-07
- [3] Larchenko E Y, Khonina T G, Shadrina E V, Pestov A V, Chupakhin O N, Menshutina N V and Chigvintsev S A 2014 *Russ. Chem. Bull.* **63** 1225-31
- [4] Larchenko E Yu, Shadrina E V, Khonina T G and Chupakhin O N 2014 *Mendeleev Commun.* **24** 201-2
- [5] Danks A E, Hall S R and Schnepf Z 2016 *Mater. Horiz.* **3** 91-112
- [6] Shadrina E V, Malinkina O N, Khonina T G, Shipovskaya A B, Fomina V I, Larchenko E Yu, Popova N A, Zyryanova I G and Larionov L P 2016 *Russ. Chem. Bull.* **64** 1633-9
- [7] Malinkina O N, Sobolev A M and Shipovskaya A B 2016 *BioNanoSci* **6** 157-61

- [8] Shipovskaya A B, Malinkina O N, Zhuravleva Yu Yu and Rogacheva S M 2016 Synthesis of silicon-containing chitosan hydrogels in a glycolic acid medium *Adv. Mater.Sci. Eng.* ID 3951703(2016)
- [9] Bianchera A, Salomi E, Pezzanera M, Ruwet E, Bettini R and Elviri L 2014 Chitosan hydrogels for chondroitin sulphate controlled release: an analytical characterization *J. Anal. Methods Chem.* ID 808703(2014)
- [10] Shipovskaya A B, Zudina I V, Fomina V I and Malinkina O 2015 *Butlerov Commun.* **41** 82-94
- [11] Khonina T G, Chupakhin O N, Larionov L P, Boyakovskaya T G, Suvorov A L and Shadrina E V 2008 *Pharm. Chem. J.* **42** 609-13
- [12] Khonina T G, Safronov A P, Shadrina E V, Ivanenko M V, Suvorova A I and Chupakhin O N 2012 *J. Colloid Interface Sci.* **365** 81-9
- [13] Wang D, Romer F, Connell L, Walter C, Saiz E, Yue S and Jones J R 2015 *J. Mater. Chem. B* **3** 7560-76
- [14] Lee E J, Jun S H, Kim H E, Kim H W, Koh Y H and Jang J H 2010 *J. Mater. Sci. Mater. Med.* **21** 207-14
- [15] Drelich J and Mittal K L 2005 *Atomic force microscopy in adhesion studies* (Boston: RC Press)
- [16] Oyen M L 2014 *Int. Mater. Rev.* **59** 44-59
- [17] Drira Z and Yadavalli V K 2013 *J. Mech. Behav. Biomed.* **18** 20-8
- [18] Jun S H, Lee E J, Yook S W, Kim H E, Kim H W and Koh Y H 2010 *Acta biomater* **6** 302-7
- [19] Suryanarayana C and Norton M G 2013 *X-ray diffraction: a practical approach* (NewYork: Springer Science & Business Media)
- [20] Ramos-e-Silva M, Hexsel D M, Rutowitsch M S and Zechmeister M 2001 *Clin. Dermatol.* **19** 460-6
- [21] Kakudo N, Kushida S, Suzuki K and Kusumoto K 2013 *J. Cos. Derm.* **12** 281-6
- [22] Kovyazin V A, Boev V V, Kopylov V M, Sokol'skaya I B 2008 *Russ. J. Gen. Chem.* **78** 216-22
- [23] Cerqueira D A, Filho G R and Assuncao R M N 2006 *Polym. Bull.* **56** 475-84
- [24] Nečas D and Klapetek P 2012 *Cent. Eur. J. Phys.* **10** 181-8
- [25] Ageev E P, Vikhoreva G A, Zotkin M A, Matushkina N N, Gerasimov V I, Zezin S B and Obolonkova E S 2004 *Polym. Sci. Ser. A* **46** 1245-50
- [26] Shipovskaya A B, Fomina V I, Kazmicheva O F, Rudenko D A and Malinkina O N 2017 *Polym. Sci. Ser. A* **59** 330-41