

NEW METHOD OF OBTAINING GELATINE-SiO₂ COMPOSITE SYSTEMS USING 3-GLYCIDOPROPYLTRIMETHOXYSILANE AND 3-CHLOROPROPYLTRIMETHOXYSILANE

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Composite materials based on biopolymers are an attractive alternative to conventional composites because of their high biocompatibility, biodegradability and unique functional characteristics. The method developed for the synthesis of material using glycidoxypropyltrimethoxysilane and chloropropyltrimethoxysilane is based on sol-gel processes making possible enhanced control over the final properties of the composite, such as water absorption and the hydrophobic or hydrophilic properties. Thermal studies of the composite show significant complexity of the formation process of the composite, and the possibility of the controlled carbonization into ceramic material.

INTRODUCTION

Composite materials based on biopolymers are becoming increasingly popular. They owe it to their properties such as biodegradability and biocompatibility. They have the possibility of widespread use, from the substitute of tissue or bone, through the electronics, catalysis, functional coatings and matrices for the immobilization of enzymes [1-3]. They are also systems for potential use for prolonged release of drugs, vitamins, extracts, and other bioactive substances from the gel to tissues, e.g. skin.

Hybrids produced by combining natural polymers with preceramic materials allow to obtain biodegradable and bioactive composite with high application ability. Materials containing chitosan, chitin or gelatine are undergoing clinical trials [4, 5]. Sol-gel method is the convenient preparation route of bioceramic composites [6-11], which allows groups of Si-O to be introduced into the organic material, for example using polydimethylsiloxane or polymethylmethacrylate, and impart to the bioactivity of the compounds [10, 11]. SiO₂ particles owe their great popularity from their optical, electrical or thermal properties. This is due to the possibility of regulating the porosity at the preparation stage, allowing the production of materials with a large amount of the active sites or high specific surface area or pore volume [12].

The process of sol-gel synthesis involves the hydrolysis and condensation reactions, which rate is controlled by the selection of the pH of the reaction medium. The process may be carried out in an organic solvent or an aqueous medium and the substrates are alkoxides (e.g. tetraethoxysilane). The reaction products have in its structure Si-O-Si bonds. The aging process during which the product particles undergo polymerization and crosslinking results in a spatial three-dimensional structure of gel. It is characterized by high viscosity that depends on the amount of substrate and solvent, temperature and time of the process. The degree of crosslinking affects the resulting porosity of the material. The way of drying and removing the

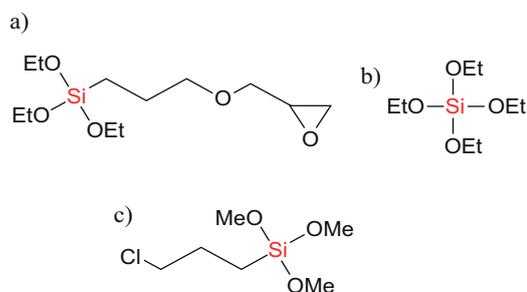


Figure 1. Structural formula of: a) (3-Glycidyloxypropyl)trimethoxysilane (Silanil 258); b) TEOS (Si-40); c) (3-chloropropyl)trimethoxysilane (3-Cl).

solvent determines the final form of the product, which is aerogel or xerogel. In case of using a supercritical drying aerogels are formed, but under conditions of reduced pressure and temperature xerogels are produced [13].

One of the most popular silanes used in this method are 3-glycidoxypropyl (BRB, Silanil 258), wherein the gelatine is dissolved with the addition of hydrochloric acid [14]. Tetraethoxysilane (BRB, Si-40 – TEOS) can also be applied for this type of synthesis. The result is a material that can potentially be used for the regeneration of bones [15].

The study presents a new synthesis method of a gelatine-SiO₂ composite systems, which involved modification of gelatine from bovine skin with: (3-chloropropyl)trimethoxysilane, 3-glycidoxypropyltrimethoxysilane (Silanil 258 BRB B.V.) and tetraethoxysilane (Si-40 BRB B.V.). SEM, TGA, water absorption test and contact angle measurements were used for characterization of the produced systems.

EXPERIMENTAL

Materials

Base gel

To a 2000 cm³ round bottom flask equipped with a thermometer and magnetic stirrer 65 cm³ ethyl silicate (Si-40, TEOS), 3 cm³ of concentrated HCl, 35 cm³ of 80 % acetic acid, 1 cm³ of poly(ethylene glycol) (PEG, molecular weight 1900-2200, Sigma Aldrich) and 600 cm³ of water was added. The solution was stirred for 12 hours and heated at 90°C. Then 35 cm³ of (3-chloropropyl)trimethoxysilane was added, continuing heating and stirring 6 hours. Subsequently, 200 cm³ of water and 10 cm³ PEG was added. The resulting 3-Cl base gel was used to obtain the following systems.

3Cl series gelatine gel

The resulting base gel was divided into two parts. 33 g of gelatine (bovine skin, Aldrich) was added to the first part of the gel, stirred using a magnetic stirrer and heated for 15 minutes at 60°C. Part of the resulting gel (225 g) was poured into a Petri dishes (3-Cl 1GEN). 100 cm³ of water and 16 g of gelatine was added to the remaining gel. The prepared slurry was stirred for 15 minutes with a magnetic stirrer and heated to 60°C. 210 g of the gel (3-Cl 2GEN) was taken from the resulting solution. In the last step 10 cm³ of polypropylene glycol (Solvadis A11/1800) was added and stirred 15 minutes with a magnetic stirrer while maintaining the temperature at 60°C. 341 g of gel (3-Cl 3GEN) was taken and dried at the room temperature and humidity of 30 % till constant weight.

3Cl EPOX series gelatine gel

To the remaining portion of the base gel 100 cm³ of water, 30 cm³ TEOS (Si-40) and 15.4 g gelatine was added. The gel was heated for 15 minutes at 60°C and stirred with a magnetic stirrer. 223 g of gel (3-Cl 1GEN EPOX) was taken for drying as described above. Next, 100 cm³ of water and 33 g of gelatine was added, stirred and heated for 15 minutes at 60°C. 154 g of the gel (sample 3-Cl 2GEN EPOX) was taken. 10 cm³ of Solvadis A11/1800 PEG was added to the residual solution, stirring and heating at 60°C for 15 minutes. 260 g of gel (3-Cl 3GEN EPOX) was taken and dried at the room temperature and humidity of 30 % till constant weight.

Extraction (EPOX E series)

To prevent the contraction of the gel caused by the capillary forces during drying, the solvent was extracted from EPOX E series gels. The wet gel was placed in a 1:1 mixture of acetone and methanol (20 cm³ of mixture per gram of the gel). The gel was left for 72 hours to remove the solvent. The following samples were obtained: 3-Cl 1GEN E, 3-Cl 2GEN E and 3-Cl 3GEN E.

Characterization of the obtained gels

Observations of gelatine-SiO₂ composites surface and fracture of surface was performed with SEM electron microscopy (Quanta 250 FEG, FEI).

Thermogravimetric analysis were performed under air flow (60 cm³·min⁻¹) from room temperature to 1223 K at heating rate of 25 K·min⁻¹ on a TG 209 F1 Libra apparatus (Netzsch Gerätebau GmbH).

Measurements of the water surface contact angle of the resulting materials were performed at room temperature and under atmospheric pressure using a Krüss DSA 100 goniometer. The volume of the dosed water drop was 10 µl.

Water absorption test was carried out as follows: the gel sample was weighed, placed in a sealed vessel with distilled water and thermostated for 24 hours at 313 K. The sample was weighed again and water absorption was calculated.

RESULTS AND DISCUSSION

Study of the morphology of composites was performed using scanning electron microscopy and results are illustrated in Figure 2. Comparing similar systems of 1st and 3rd generation can be noticed that the systems containing epoxysilane modifier possess more compact structure. This confirms the assumption of a higher degree of cross-linking. In case of the series modified with one type of silane (Figure 2c, d, g, h), the presence of pores in the microscopic structure and significant differences in the surface morphology are observed (Figure 2d, h).

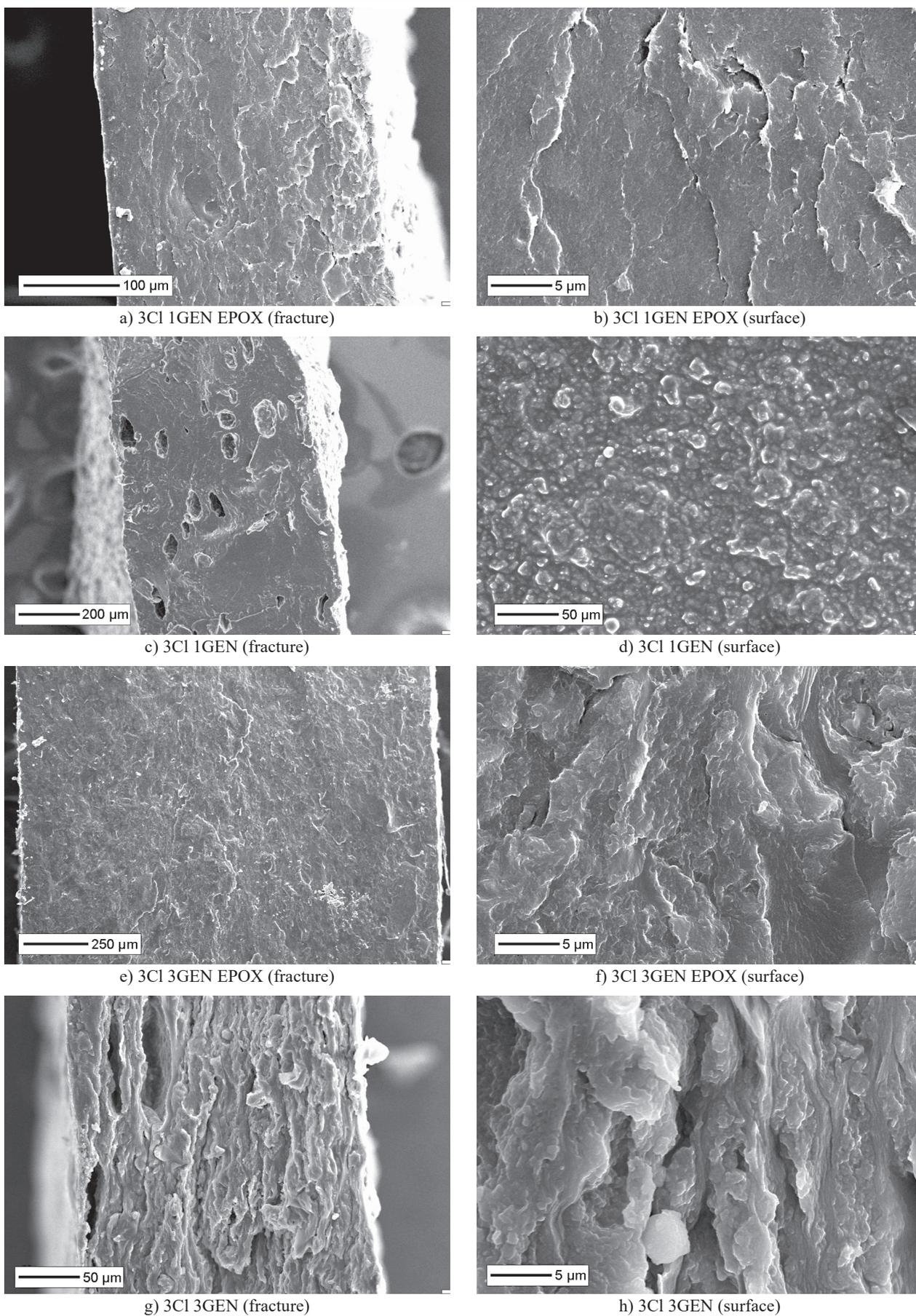


Figure 2. SEM photos of gelatine-silane composites.

The results of thermal analysis of gelatine-SiO₂ composites are shown in Figure 3 and 4. Under nitrogen atmosphere, the thermal decomposition takes place in three steps (Figure 3). The first weight loss occurring below 200°C is associated with the removal of physically bound water and volatile organic compounds used in the synthesis (alcohols, carboxylic acids); at about 300°C is due to the carbonization and degradation of the gelatine proteins [16], and above 400°C decomposition of silanes in the gel network [17]. Processes is accompanied by release of gases (H₂O, CO, CO₂, NH₃) in areas of strong cross-linking of the gel.

For each system, strong exothermic effect above 500°C accompanying continuous weight loss occurs. Maximum of this exothermic effect appears in the range of 570°C, however in case of 3GEN sample it is mostly shifted towards temperature exceeding 600°C with maximum at about 700°C. 1GEN EPOX sample exhibits also smaller positive thermal effect at about 650°C, probably related to the further silane component transformation.

In an air atmosphere a four-step decomposition of gelatine-SiO₂ systems takes place (Figure 4). In this case the additional loss of mass associated with oxidation and breakdown of gelatine proteins above ca 600°C occurs [17]. The aforementioned strong exothermic effect exists above ca 600°C with maximum at about 630°C and additionally is correlated with mass loss (DTG / DTA peak shape), except of 1GEN EPOX sample (slower weight loss). Moreover, only in case of 3GEN sample, it is accomplished just about 650°C with the stop of weight loss. Strong thermal effect above 600°C and significant mass loss at this stage, on the contrary to the nitrogen atmosphere, result from oxidation of organic components and synthesis residues (gelatine, silane, and other organic remainings). Onset of constant weight stabilization indicates that mostly refractory inorganic components (silica) remain in the samples.

For comparison, the TGA study of the thermal decomposition process of unmodified gelatine is presented on Figure 5 [16]. The presence of the silane affects the thermal resistance by the decomposition temperature

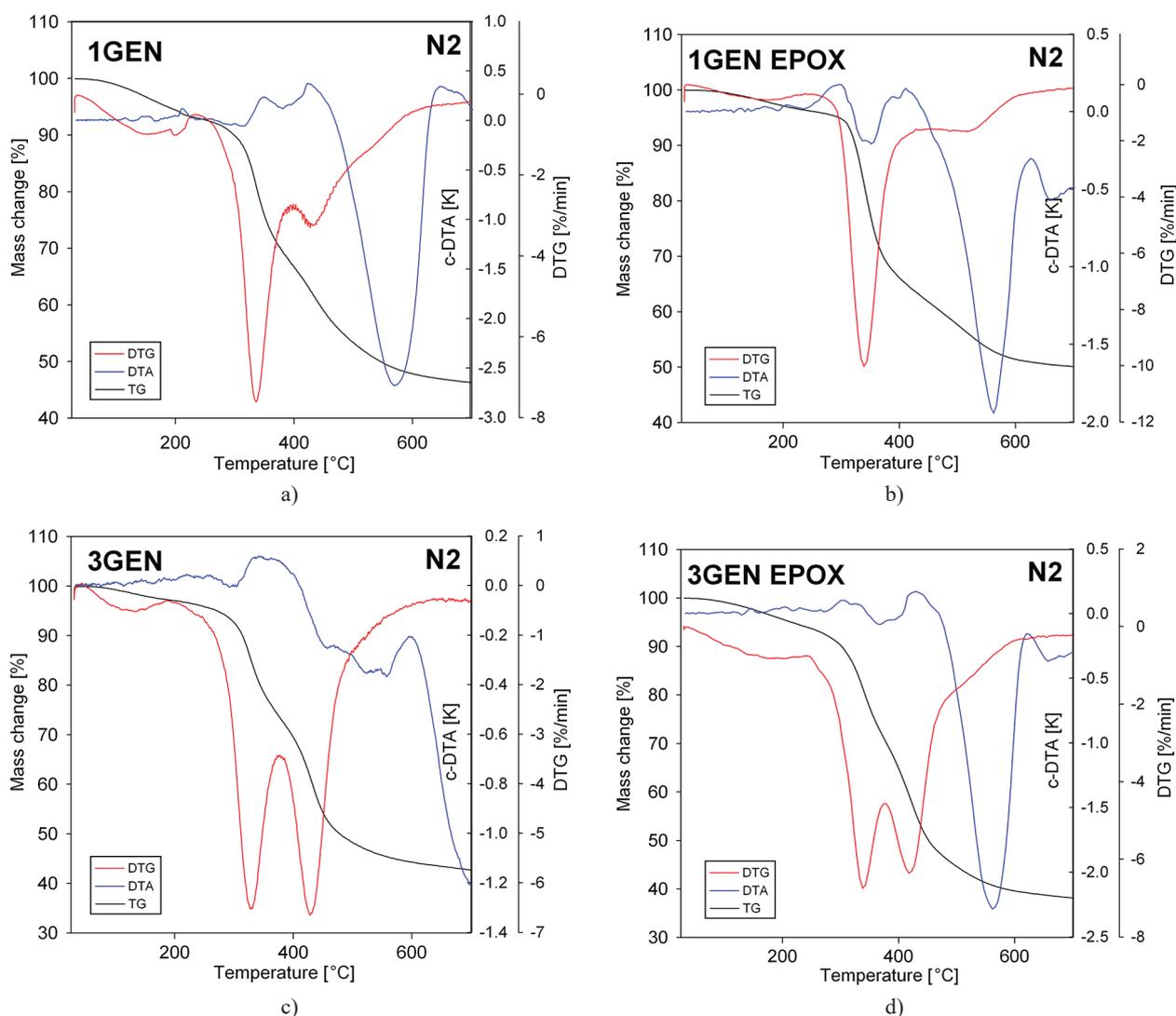


Figure 3. Thermal analysis of gelatine-SiO₂ composite in the nitrogen atmosphere.

increase both of the composite and the gelatine protein component.

To test the adhesive properties of the resulting gels wetting angle measurements were performed. Hybrid systems and gelatine reference sample were examined using a Krüss DSA 100 goniometer. The measurement allows to determine the contact surface contact angle for

the sample along with the registration of the shape of the drop with a digital camera. The results of the surface wetting angle measurements of water are summarized in Table 1.

Table 1. Results of surface wetting contact angle measurements.

Sample	Wetting angle [deg]	Properties
3Cl 1GEN	114	hydrophobic
3Cl 2GEN	107	hydrophobic
3Cl 3GEN	80	hydrophilic
3Cl 1GEN EPOX	79	hydrophilic
3Cl 2GEN EPOX	48	hydrophilic
3Cl 3GEN EPOX	diffused drop	hydrophilic
3Cl 1GEN EPOX E*)	61	hydrophilic
3Cl 2GEN EPOX E*)	59	hydrophilic
3Cl 3GEN EPOX E*)	66	hydrophilic

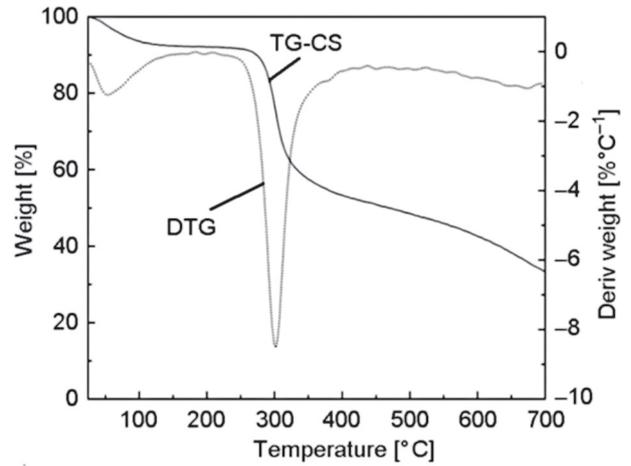


Figure 5. Thermogram and DTG curves of gelatine [16].

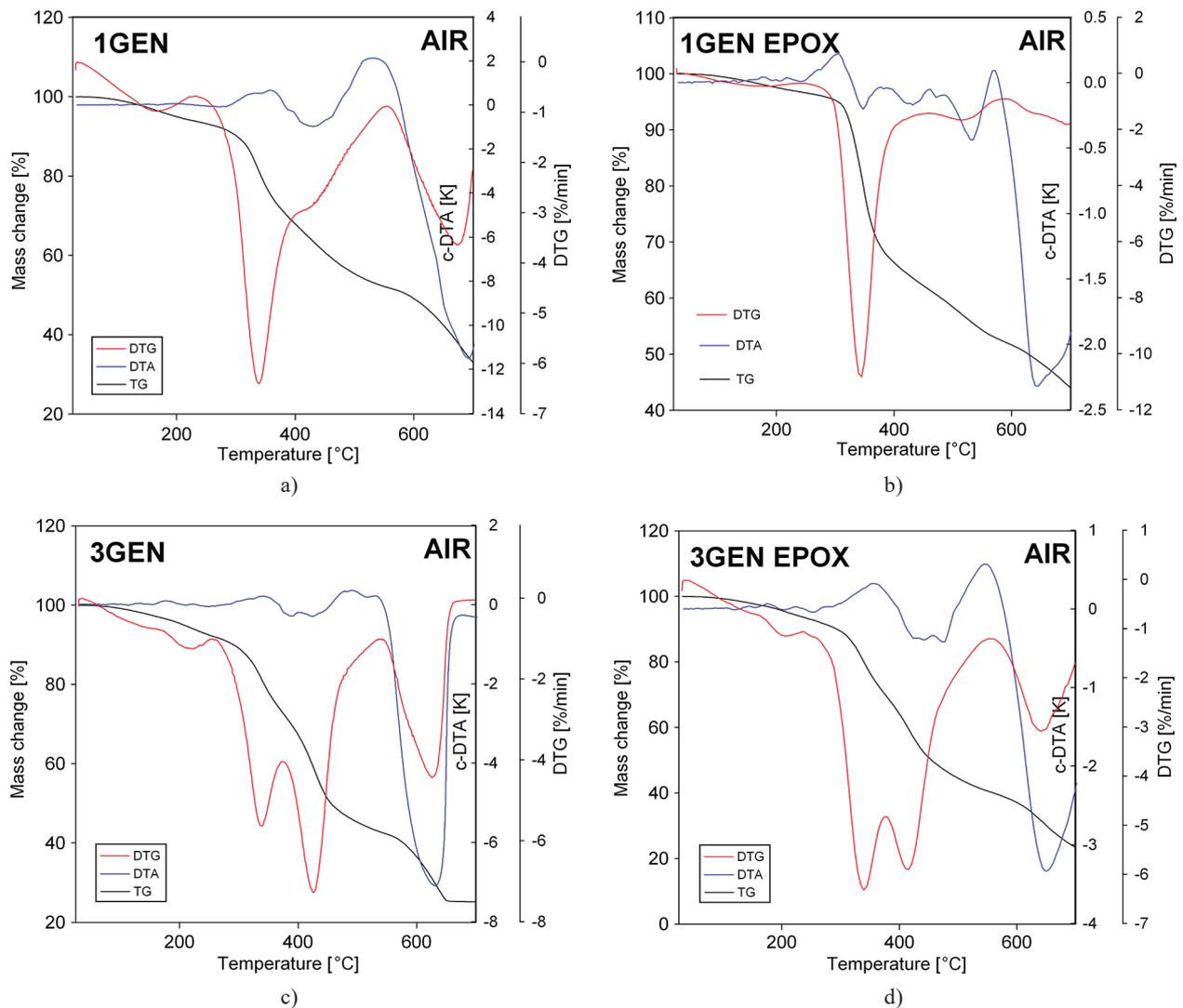


Figure 4. Thermal analysis gelatine-SiO₂ composite in the air atmosphere.

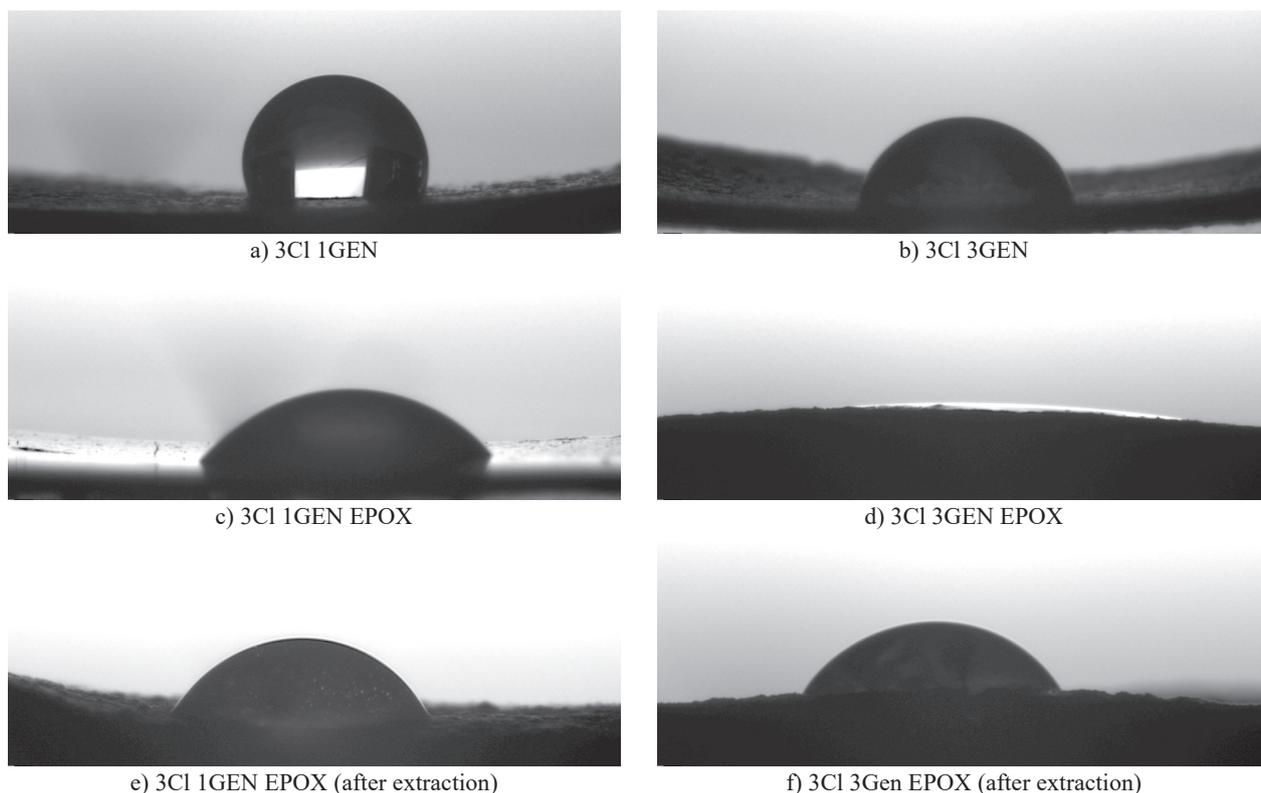


Figure 6. Images of water drops on the samples surface.

The surface is hydrophobic when the contact angle is in the range of 90 - 120° and hydrophilic properties are characterized by the contact angle is below 90°. With increasing amount of the gelatine the surface wettability increases, which is reflected by the value of the measured surface contact angle.

A decrease of the hydrophobic character with the increasing protein content of the composite is observed in each series. The addition of epoxysilane decreases the hydrophobic character as compared to a similar sample in the 3-Cl series. At the same time, it can be seen that the samples after solvent extraction are more hydrophobic compared to their counterparts dried in a conventional way.

The water absorption of the biopolymers composites is an important parameter in view of their use as a support matrix for controlled release system of drugs or plant nutrients [18, 19].

The results of water absorption tests are shown in Figures 7-9 and summarized in Table 2.

The highest water absorption is observed for the first series of composites, then for the series after the extraction of water. The weakest sorption show the epoxysilane modified systems (the second series). With the increasing content of gelatine additive the water absorptivity in the series increases. It appears that this effect may be related both to the hydrophilic nature of the gelatine proteins and less compact structure of the

Table 2. Results of water absorption tests.

Sample	Absorption after ¹⁾ :					Remarks
	1 h	2 h	3 h	4 h	24 h	
3Cl 1GEN	163	167	168	170	178	elastic
3Cl 2GEN	142	166	182	193	242	swollen, brittle
3Cl 3GEN	151	179	202	221	298	swollen, brittle
3Cl 1GEN EPOX	109	111	111	111	113	brittle
3Cl 2GEN EPOX	114	118	128	133	142	swollen, brittle
3Cl 3GEN EPOX	117	125	134	139	152	swollen, brittle
3Cl 1GEN EPOX E ²⁾	171	174	178	178	179	swollen, brittle
3Cl 2GEN EPOX E ²⁾	168	169	171	171	175	swollen, brittle
3Cl 3GEN EPOX E ²⁾	126	137	145	150	159	swollen, brittle

¹⁾ [% w/w]; ²⁾ Samples after extraction

silane-gelatine hybrids visible in SEM micrographs. Compared to their air-dried counterparts, the systems that undergo a solvent extraction process are significantly less hydrophilic nature of the surface while increasing the sorption capacity with respect to water. This may be caused by the preservation of the original structure of the wet gel that does not shrink or collapse under capillary forces accompanying evaporative drying of the gel [18, 19].

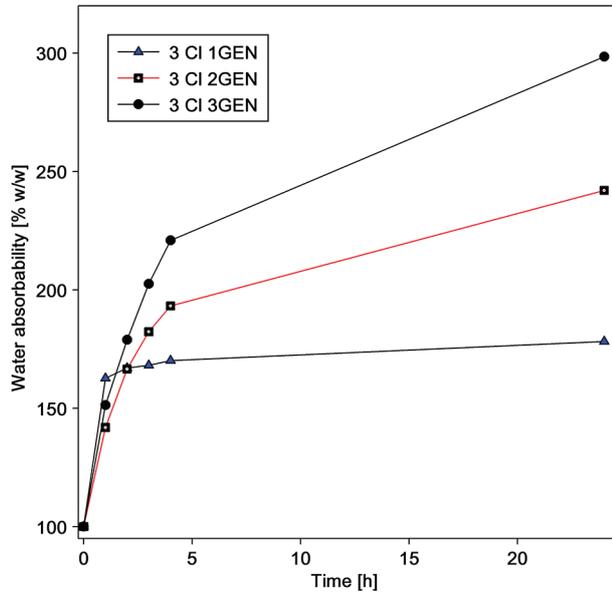


Figure 7. Water absorbability of 3Cl series samples as a function of time.

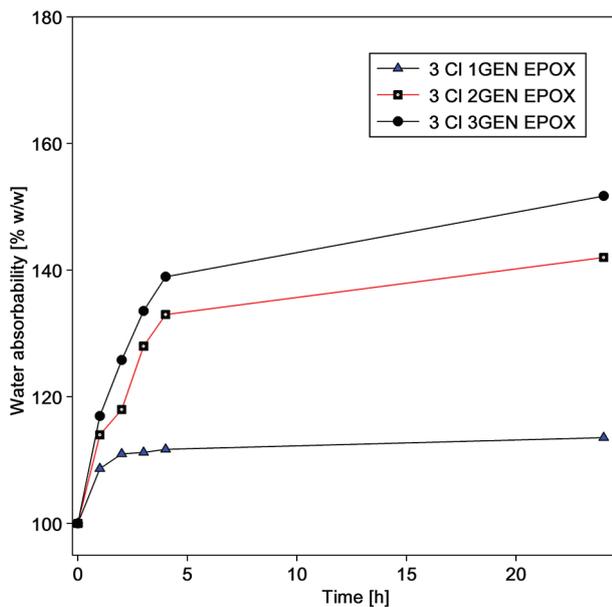


Figure 8. Water absorbability of 3Cl EPOX series samples as a function of time.

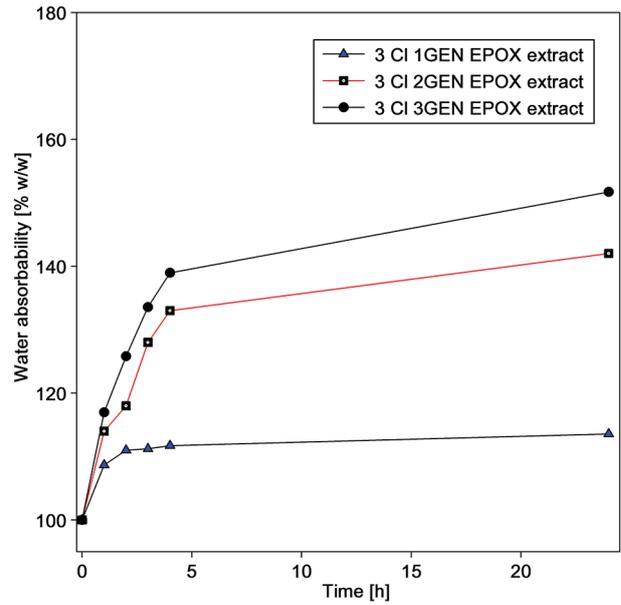


Figure 9. Water absorbability of EPOX E series samples as a function of time.

CONCLUSIONS

Water absorption test indicate that systems with products hydrolysis and condensation of TEOS and 3-Cl silane show the highest absorption of water, despite the fact that their surface is hydrophobic. This is caused by a lower degree of the material crosslinking than the systems containing Silanil 258 silane as third component.

The structure of systems with Silanil 258 silane content is more compact and rigid because of higher degree of cross-linking caused by the presence of highly reactive epoxy group in the silane molecule, and thus the absorption is reduced. The surface of these materials is hydrophilic.

By using a combination of factors as the type and amount of silane in the hybrid material and by the selection of the gel drying method, both the nature of the hydrophobic-hydrophilic composite surface and its ability to absorb water can be influenced. An additional advantage of the proposed method is the possibility of design a material that will be, on the one hand, characterized by a high hydrophobicity of surface and, on the other hand, will have a high bulk capacity of water absorption.

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REFERENCES

1. Draye J. P., Delaey B., Van de Voorde A., Van Den Bulcke A., Bogdanov B., Schacht E. (1998): *In vitro* release characteristics of bioactive molecules from dextran dialdehyde cross-linked gelatin hydrogel films. *Biomaterials*, 19, 99-107. doi: 10.1016/S0142-9612(97)00164-6
2. Hench L.L. (1991): Bioceramics: From Concept to Clinic. *Journal of American Ceramic Society*, 74, 1487-1510. doi: 10.1111/j.1151-2916.1991.tb07132.x
3. Kokubo T. (1991): Recent progress in glass-based materials for biomedical applications. *Journal of the Ceramic Society of Japan*, 99, 965-973. doi: 10.2109/jcersj.99.965
4. Tabata Y., Ikada Y. (1999): Vascularization effect of basic fibroblast growth factor released from gelatin hydrogels with different biodegradabilities. *Biomaterials*, 20, 169-2175. doi: 10.1016/S0142-9612(99)00121-0
5. Zhang Q. Q., Liu L. R., Ren L., Wang F. J. (1997): Preparation and Characterization of Collagen-Chitosan Composites. *Journal of Applied Polymer Science*, 64, 2127-2130.
6. Yamada N., Yoshinaga I., Katayama S. (1999): Effect of inorganic components on the mechanical properties of inorganic-organic hybrids synthesized from metal alcoxide and polydimethylsiloxane. *Journal of Material Science*, 14, 1720-1726. doi: 10.1557/JMR.1999.0233
7. Philipp G., Schmidt H. (1984): New materials for contact lenses prepared from Si and Ti alcoxides by the sol-gel process. *Journal of Non-Crystalline Solids*, 63, 283-292. doi: 10.1016/0022-3093(84)90407-1
8. Mackenzie J.D., Hu Y. (1992): Rubber-like elasticity of organically modified silicates. *Journal of Material Science*, 27, 4415-4420. doi: 10.1007/BF00541574
9. Guo L., Lee J.H., Beaucage G. (1999): Structural analysis of poly(dimethylsiloxane) modified silica xerogels. *Journal of Non-Crystalline Solids*, 243, 61-69. doi: 10.1016/S0022-3093(98)00819-9
10. Chen Q., Miyaji F., Kokubo T., Nakamura T. (1999): Apatite formation on PDMS-modified CaO-SiO₂-TiO₂ hybrids prepared by sol-gel process. *Biomaterials*, 20, 1127-1132. doi: 10.1016/S0142-9612(99)00015-0
11. Tsuru K., Ohtsuki C., Osaka A., Iwamoto T., Mackenzie J.D. (1997): Bioactivity of sol-gel derived organically modified silicates. *Journal of Material Science: Materials in Medicine*, 8, 157-161. doi: 10.1023/A:1018523203667
12. Hernandez M. A., Velasco J. A., Asomoza M., Solis S., Rojas S., Lara V.H. (2004): Adsorption of benzene, toluene, and p-xylene on microporous SiO₂. *Industrial & Engineering Chemistry Research*, 43, 1779-1787. doi: 10.1021/ie0204888
13. Brinker C. J., Scherer G. W. (1990). Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing, Chapter 8. Academic Press. pp. 453-515.
14. Smitha S., Mukundan P., Krishna Pillai P., Warriar K. G. K. (2007): Silica-gelatin bio-hybrid and transparent nano-coatings through sol-gel technique. *Materials Chemistry and Physics*, 103, 318-322. doi: 10.1016/j.matchemphys.2007.02.068
15. Brinker C. J., Scherer G. W. (1990). Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing, Chapter 1. Academic Press. pp. 1-21.
16. Qi M. J., Qian Y. F., Zhao Y. P., Wei J. (2015): Thermal Behaviour of Electrospun Gelatin and Chitosan Complex. *Thermal Science*, 19(4), 1323-1326. doi: 10.2298/TSCI1504323Q
17. Pietras P., Przekop R., Maciejewski H. (2013): New approach to preparation of gelatine/SiO₂ hybrid systems by the sol-gel process. *Ceramics – Silikaty*, 57 (1), 58-65.
18. Stavinskaya O., Laguta I., Orel I. (2014): Silica-Gelatin Composite Materials for Prolonged Desorption of Bioactive Compounds. *Material Science*, 20 (2), 171-176. doi: 10.5755/j01.ms.20.2.4966
19. Stavinskaya O., Laguta I., Kuzema P. A. (2011): Effect of Highly Dispersed Silica on Water Absorption of Gelatin Materials. *Protection of Metals and Physical Chemistry of Surfaces*, 47(30), 302-306. doi: 10.1134/S2070205111030154