NEW APPROACH TO PREPARATION OF GELATINE/SiO_2 HYBRID SYSTEMS BY THE SOL-GEL PROCESS

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A new method of synthesis of a series of hybrid gelatine/silica systems, based on solgel technique, was developed in this study which was aimed at achieving the maximum compatibility of the gelatine/silica systems prepared in a wide range of gelatine-silica ratios (from 30 to 80 wt. % of gelatine). The systems were characterized by X-ray diffraction, SEM, TEM, FT-IR, and TG techniques. The obtained materials showed a diverse structure due to the fact that a part of silica formed a homogeneous composite with gelatine, while another part formed microspherical particles of complicated structure. A possibility was tested for employing branched polyalkoxysiloxanes as crosslinkers in the preparation of gelatine-silica hybrids.

INTRODUCTION

Hybrid materials are designed with highly tailorable properties, which are achieved through careful control of synthesis parameters. These materials have great potential for biotechnological, biomedical and pharmaceutical applications, particularly as materials of high degree of compatibility with biological materials and as matrices for encapsulation of bioactive substances. The design of metal oxide/polymer hybrid systems has attracted much attention in the last few years, due to their wide range of applications. Biopolymer/silica hybrid materials are composites inspired by nature that permit to obtain a high compatibility with tissues and systems of biological origin. According to the literature, gelatine/silica hybrids can find applications in bone regeneration strategies, angiogenesis, drug delivery and cements [1], as well as in optical coatings, high refractive index films, contact lenses, thin film transistors, light emitting diodes, solar cells, optical waveguide materials and photochromic materials, adsorption, catalysis and high energy fields [2].

Many of these nanocomposites were designed as polymer-core/oxide-shell materials [3-5], although oxide-core/polymer-shell [6-8] as well as bicontinuous structures were also described [9-11]. It has been shown that gelatine, both in solution or as thin films [12-13], could activate silica formation and this property was employed for designing biomimetic silica-coated gelatine macrobeads. [14]. One of the most convenient methods for preparing silica/gelatine systems that provides with a wide range of possibilities, is hydrolysis and condensation of silica precursors in conditions of controlled pH (sol-gel method). Biocomposites prepared by using the above method have exceptional properties such as high plasticity, low modulus of elasticity and high strength [2, 15-17]. The above method enables to obtain materials of the type core/shell gelatine/silica or silica/gelatine, depending on biopolymer concentration, pH of solution and silica precursor concentration [18].

In the case of hybrid materials, of great importance is the formation of durable bonds between biopolymer and inorganic material. The essential role in the formation of such bonds is played silane coupling agents such as amino and epoxy functional silanes, etc. [19]. The coupling agent chosen most frequently for gelatine-containing systems was 3-glycidoxypropyltrimethoxysilane [20-23].

In this paper we have presented syntheses of hybrid materials aimed at obtaining possibly the highest compatibility degree of systems prepared which was achieved by integrating two types of polymers: organic biopolymer (gelatine) and inorganic polymer (silica gel). The essential difference between our synthesis procedure and that employed in earlier studies on systems functionalized in such a way is the application of U-740 ethyl silicate (that is a hydrolyzed and partly polymerized form of a simple ester such as tetraethyl orthosilicate) as a silica precursor. The application of the above compound is based on the conjecture that it can have an advantageous influence on hybrid system compatibility. In order to verify this conjecture, hydrolysis and condensation of ethyl silicate and 3-glycidoxypropyltrimethoxysilane (GPTMS) were performed and product of the above reactions, namely functionalized silica sol, was subjected to reaction with gelatine. The product of the latter reaction was characterized by using XRD, TEM, SEM, TG and FT-IR techniques.

EXPERIMENTAL

Chemicals

Gelatine employed in the study originated from porcine skin (type A, isoelectric point (pI) = 8, 300 Bloom, dry matter 90 %) was purchased from Aldrich. Ethyl silicate - U740 (40 % SiO_2) and 3-glycidoxypropyltrimethoxysilane (GPTMS) were obtained from UNISIL and glacial acetic acid from Aldrich.

Silica/gelatine hybrid systems

The starting sol was obtained by hydrolysis and condensation of an appropriate amount of ethyl silicate (U740, $d = 1.05$ g/cm^3, 40 % SiO_2) and GPTMS in water in the presence of acetic acid as pH control agent. The amount of silica formed as a result of hydrolysis and condensation of both precursors was taken into account each time when proportions of ethyl silicate to 3-glycidoxypropyltrimethoxysilane were chosen. Weight ratio of $SiO_{2\ U740}$ to $SiO_{2\ GPTMS}$ was constant and equal to 4:1. Mole ratio of $SiO_{2\ U740}$:H_2O:CH_3COOH was 1:77:1.2. The mixture was vigorously stirred and heated at 358 K for 6 h to yield functionalized silica gel. Codes and compositions of samples obtained are shown in Table 1. At the next stage, gelatine was added to the obtained sol and the mixture was heated at 333 K for 2 h with vigorous stirring. After that time, the obtained mixture was placed on plastic Petri dishes and left covered for 96 h for gelation and ageing. Then covers were removed and the material was dried at room temperature for 2 weeks.

Characterization of the systems

The obtained materials were characterized by using XRD, TEM, SEM and FT-IR techniques.

The X-ray powder diffraction patterns of samples were taken on a Philips PW1050 diffractometer, with Ni-filtered CuK_α radiation. The surface morphology of the hybrids was examined on a scanning electron microscope (Philips 515 SEM) and structures of the hybrids were characterized using a transmission electron microscope (JEOL 200 CX). Stereoscopic pictures were taken using an Olympus SZX-7 microscope. FT-IR spectra were recorded in the wavenumber range 500 - 4000 cm^{-1} on a Bruker Tensor 27 Fourier transform spectrometer equipped with a SPECAC Golden Gate diamond ATR unit. In all cases 16 scans at a resolution of 2 cm^{-1} were collected for the spectrum. Thermogravimetric analysis was performed under air flow (60 cm^3/min) from room temperature to 1223 K at heating rate of 25 K/min on a Q50 apparatus (TA Instruments).

RESULTS AND DISCUSSION

Samples morphology

To obtain silica/gelatine hybrid, where covalent bonds are present, it is necessary to employ a coupling agent which plays the role of a linker between organic and inorganic material. In this study, silane coupling agent, such as 3-glycidoxypropyltrimethoxysilane (GPTMS), was first incorporated into silica sol structure and then the sol was modified with gelatine, which is an approach different from those reported in the literature [1]. Moreover, ethyl silicate, subjected to preliminary polymerization (SiO_2 content: 40 %), was employed as a silica precursor. Ethyl silicate is used as a crosslinker for hydroxylated synthetic polymers such as vinyl chloride/vinyl acetate copolymers, vinyl chloride/vinyl acetate/hydroxyalkyl acrylate copolymers and others. According to the literature, the application of the above crosslinker results in an improvement in the resistance to solvents, flexibility, hardness and other mechanical properties due

Table 1. Composition of materials prepared.

Sample code	Weight of SiO_2 (g) originated from U740	Weight of SiO_2 (g) originated from GPTMS	Weight of gelatine (g)	SiO_2 content (%)	Thickness of spherical SiO_2 layer (% of total thickness)
G0	42.0	10.5	-	100.0000	-
G1	38.0	9.5	18.00	70.0	47
G2	32.8	8.2	33.62	52.0	28.5
G3	27.3	6.85	46.50	39.9	26.0
G4	23.2	5.8	56.16	31.1	24.1
G5	18.0	4.5	61.88	24.3	15.4
G6	13.2	3.3	62.55	18.6	12.6

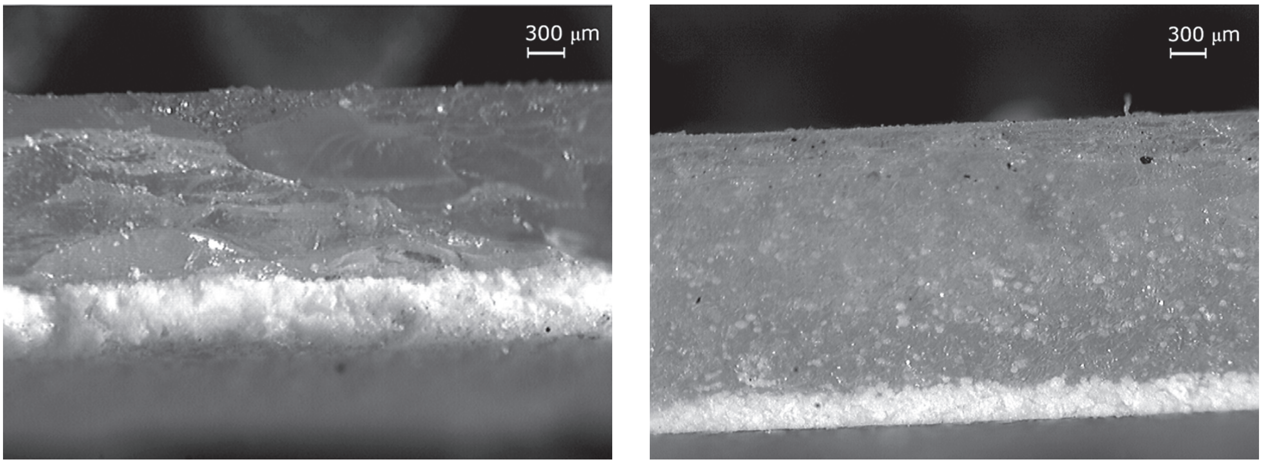
to crosslinking of hydroxyl groups in the structure of organic polymer [24]. This was why we have used it as a precursor of inorganic component of the hybrid material. The syntheses carried out by us resulted in obtaining hybrid gels that assumed the shape of a mold they were poured into. In each case, a characteristic feature of the prepared materials was diversification of morphology and composition of upper and lower parts of hybrid xerogels obtained (Figure 1a, b). The upper part was a transparent material of straw-yellow coloration, whereas the bottom part was white. Such a morphological diversification was observed in each case investigated. Smitha et al. [23] reported that the addition of gelatine can result in the settlement of a white silica precipitate of different morphology in silica-containing systems. The mentioned effect is a result of the fact that positively charged peptides or proteins activate silicate condensation of silicate anions [12]. This is associated with the increase in pH of the system after the addition of gelatine (isoelectric point > 7). As pH of the sol increases above pH 5, the charge on silica increases. This leads to electrostatic interactions with charged molecule of gelatine which results in salting out. In the case of our synthesis method, the settlement of a part of *Stöber Silica* particles as a white precipitate occurs already at the stage of functionalized silica sol formation, therefore it is not caused by the addition of gelatine. It is also worth to add that in the case of our method of synthesis, no pH correction was made, therefore pH depended only on amounts of reagents used by us.

Micrographs of cross-sections of the samples (Figure 1 a, b) have shown that the white precipitate, accumulated at the bottom part, consists of spherical microparticles. The *Stöber Silica* microparticles, precipitated on Petri dishes during the stage of gel ageing, sedimented and accumulated at the bottom part of the gel formed. Thickness of the bottom part of the precipitate depends on silica/gelatine ratio (Table 1).

In order to isolate the *Stöber Silica* microparticles sedimented and to perform analysis, the separation was carried out by means of hybrid sol filtration under vacuum. We have succeeded in isolating spherical particles of micrometric size (Figure 2 a, b) that were identified on the ground of XRD studies as amorphous silica. X-ray patterns (Figure 3 a) of this material were characterized by the presence of broad reflection lines at 26 - 23 2θ degrees which corresponded to amorphous silica.

Diameters of *Stöber Silica* spherical particles have fallen in the range from about 5 to 80 μm (Figure 2a,b). As gelatine content in the material increased (and consequently the increase in the system viscosity and the rate of crosslinking occurred) the mobility of silica microparticles diminished. This is why the number of SiO_2 particles that underwent a complete sedimentation diminished and a part of them was permanently immobilized in the upper part of the hybrid material (Figure 1b). Spherical silica present in the gel has a porous structure on nanometric level. The observation of mechanically damaged particles enabled to see their interior (Figure 2c). A porous nanostructure consisting of silica particles of nanometric size is shown in Figure 2d.

In further part of the study, a microscopic characterization of surfaces of hybrid xerogels was performed. As it was already mentioned, bottom and upper parts of the systems obtained differ in their morphology. The upper part of the monolith (Figure 2e) is characterized by high smoothness and cohesiveness, the absence of pores in the micrometric range, as well as the absence of fractures and kinks. No SiO_2 microparticles can be seen. The bottom part, at which an accumulation of occluded silica microspheres has occurred (Figure 2f), makes a conglomerate of spherical microparticles linked by silica-gelatine hybrid gel that is a binding material for silica spheres.

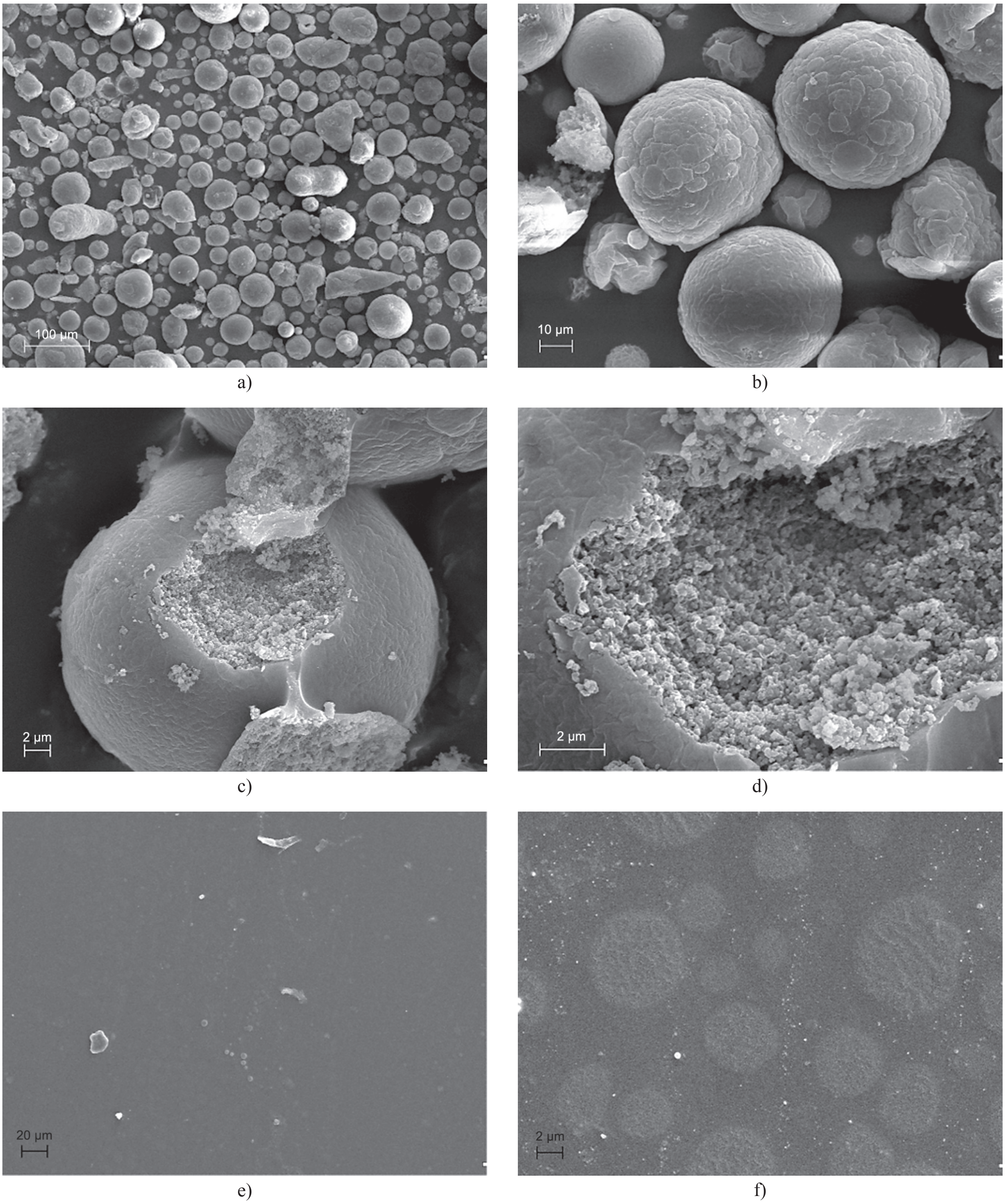


a) G3

b) G6

Figure 1. Cross-section of samples of G3 (a) and G6 (b) generations. Accumulation of *Stöber Silica* in the form of microspheres can be seen at the bottom part of the material.

On the nanometric scale (Figure 4 a, b) the presence can be seen of silica nanoparticles being a result of condensation of a part of silica material into spherical particles (dark areas in micrographs) prior to the stage of hybrid system formation (light areas in micrographs). The particles are linked to the hybrid obtained. X-ray analysis showed broad reflection lines at 26 - 23 2θ degrees (Figure 3 b-g) that corresponded to amorphous silica. For hybrid xerogels, the structure expands and in the case of samples of lower gelatine content the



a) b) c) d) e) f)

Figure 2. Scanning electron micrograph images of materials studied. (a, b) - SiO_2 microspheres isolated from material G4 by filtration, (c, d) - SiO_2 microspheres isolated from material G3 by filtration; chipped off fragment of outer part of the sphere (c) and magnified nanostructure of the interior (d) are seen, (e) - Highly homogeneous surface of the top of material G5, (f) Surface of the bottom part of material G5; spherical particles of SiO_2, bound in hybrid gel, can be seen.

peak at 26 2θ degrees shifts to lower angles. The results indicate that gelatine molecules were incorporated into the silica structure.

IR measurements

Hybrid systems that contain polymers of biological origin as their components, particularly protein polymers, make a matrix that is difficult to study on a molecular level. Complexity of amino acid composition and the presence of different functional groups in protein structure make difficult the interpretation of results of spectroscopic measurements. The reference spectrum of gelatine is characterized by a large band around 3279 cm^{-1} (Figure 5 A2) corresponding to NH stretching vibrations, narrow bands at 2920 cm^{-1} and 2850 cm^{-1} attributed to CH_2 stretching vibrations (Figure 5 B1, B2). Bands at 1631 cm^{-1} and 1524 cm^{-1} (Figure 5 C1, C2) corresponding to the amide I and II bands mainly associated with the C=O stretching vibrations (70 - 85 %), is directly related to the backbone conformation. Amide II band originates from the N–H bending vibrations (40 - 60 %) and from the C–N stretching vibrations (18 - 40 %) [25, 26]. The spectrum of GPTMS has a characteristic strong and sharp band at 1076 cm^{-1} ascribed to Si–O–C stretching vibrations (Figure 5 D1), and a band 908 cm^{-1} originated from oxirane ring vibrations (Figure 5 E2). Functionalized U740/GPTMS gel gives a broad band at 3375 cm^{-1} (Figure 5 A1) coming from stretching vibrations of hydrogen bonded and free hydroxyl groups present in xerogel. Moreover, the spectrum of U740/GPTMS system shows the presence of a band at 1051 cm^{-1} (Figure 5 E2) that is characteristic of [SiO_4] tetrahedron vibrations, bands at 954 cm^{-1} and 794 cm^{-1} originated from non-bridging oxygen vibrations in Si–OH and Si–O–Si bending vibration mode in SiO_2, respectively (Figure 5 E1).

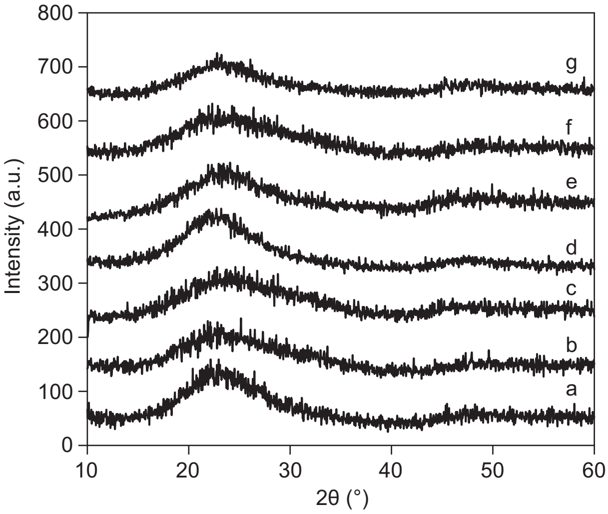
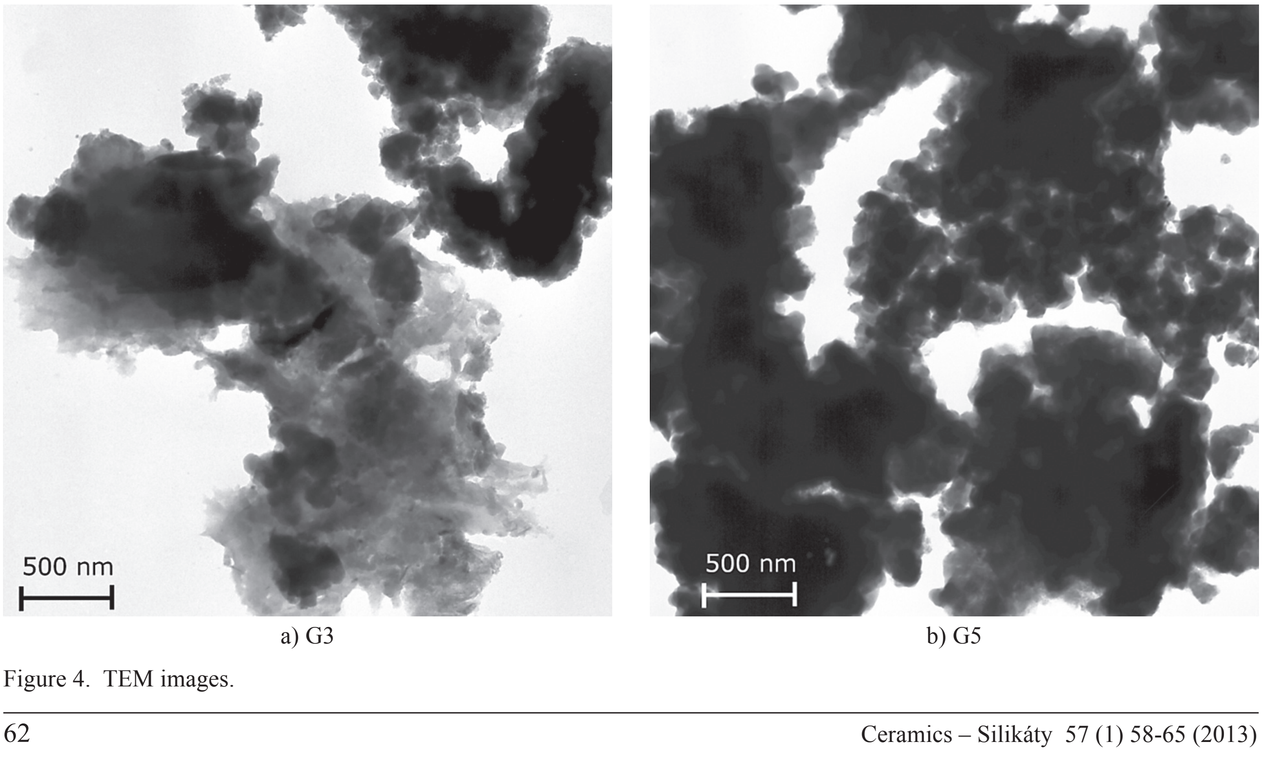


Figure 3. XRD patterns of hybrid materials a) isolated spherical SiO_2 particles; b) G1; c) G2; d) G3; e) G4; f) G5; g) G6.

Due to inhomogeneity of the structure of materials, caused by sedimentation of spherical silica microparticles, their FT-IR spectroscopic characterization was carried out using ATR technique for recording spectra of both sides of obtained hybrid xerogels (Figure 6, top and bottom). Typical silica bands were present in spectra of both sides of materials**:** the triply degenerated stretching and bending vibration modes of [SiO_4] tetrahedra at 1034 cm^{-1} (Figure 6f), Si–OH vibrations at 945 cm^{-1} (Figure 6g) and Si–O–Si bending vibrations (Figure 6h).



a) G3

b) G5

Figure 4. TEM images.

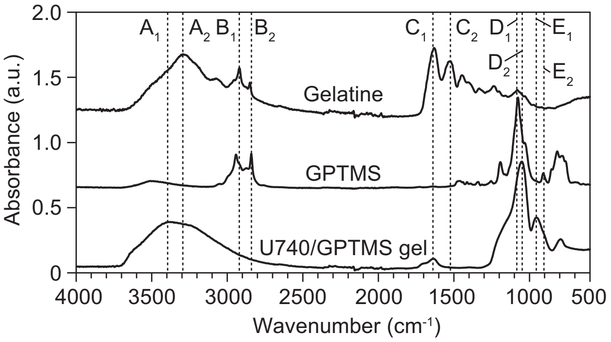


Figure 5. IR spectra of gelatine, GPTMS and functionalized silica gel U740/GPTMS.

In each case, intensities of bands characteristic of silica were higher for the top part of samples of the same generation than for the bottom part, in spite of the fact that percentage of gelatine in the upper part was higher than in the bottom part. This results from the fact that in the case of the bottom part, silica is accumulated in the form of microspheres that are porous solids (Figure 2c,d), therefore the amount of Si–O–Si groups per unit of volume is smaller and this is reflected by lower intensities of bands observed. In spectra of all series of obtained materials, a broad band at 1030 - 1070 cm^{-1} is present (Figure 6) both in the case of the upper and bottom parts. The above band is a superposition of the band at 1076 cm^{-1} (Figure 6e) originated from Si–O–C vibrations and the band at 1034 cm^{-1} from bending vibrations of [SiO$_4$] tetrahedra. As percentage of gelatine

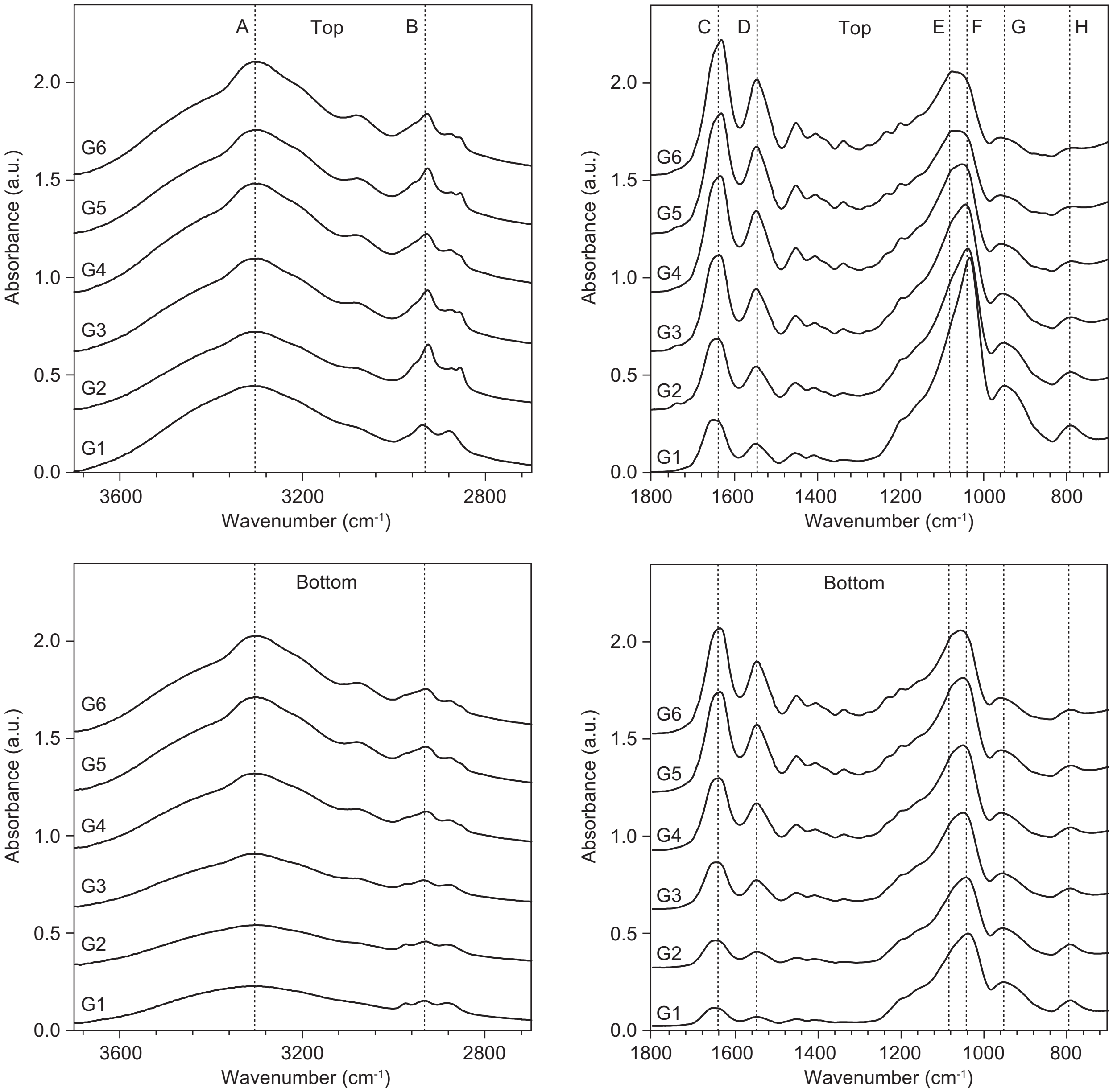


Figure 6. IR spectra of top and bottom parts of hybrid materials.

grows, intensity of the band at 1034 cm^{-1} diminishes and that of Si–O–C increases. It seems to be directly associated with growing number of links between silica gel and gelatine due to condensation of silanol groups with carboxyl and hydroxyl groups present in peptide molecule. As gelatine content grows, a proportional increase in intensities of bands characteristic of gelatine (Figure 6a-d) and a proportional decrease in intensities of bands characteristic of functionalized silica gel (Figure 6f-h) are observed. In all spectra, there are no bands originated from oxirane ring (908 cm^{-1}), which probably reacted with reactive groups (COOH, NH_2, OH) present in the system.

Thermogravimetric analysis

In Figure 7 thermogravimetric analysis profiles of silica obtained from U740, functionalized silica gel (U740/GPTMS) and gelatine are shown for reference purposes. Non-functionalized silica shows a peak at 374 K originated from the removal of physically adsorbed water and residue of organic compounds left after precursor hydrolysis (Figure 7). In the case of functionalized system GPTMS, a peak with a maximum at 620 K is observed that should be ascribed to the decomposition of epoxy propyl groups. Unmodified gelatine decomposes at three stages the first of which (< 374 K) originates from the evolution of physisorbed water. The second stage that ranges from 500 - 700 K reflects degradation of protein structure and evolution of volatile products resulting from its carbonization. The third stage, observed above 800 K, is associated with oxidation of the carbonizate formed at the second stage. Thermogravimetric analysis curves of hybrid materials (Figure 8), in spite of molecular complexity of the matrix, are a superposition of curves recorded for reference materials and permit to discern three stages. The first in the range up to 530 K is associated with the removal of physisorbed water and volatile organic compounds bound in the gel matrix. Sharp peaks in the range 450 - 550 K are a result of a violent evolution of volatile compounds introduced or formed during synthesis (alcohols, carboxylic acids). The second stage (from 580 K to 750 K) can be ascribed to the decomposition of protein component of the system and its carbonization. The presence of small sharp peaks in thermal analysis curves originates most likely from the release of gases (H_2O, CO, CO_2, NH_3) formed in the areas of strong crosslinking of the gel. The third stage (750 - 960 K) corresponds to oxidation of carbonizate formed as a result of protein decomposition. The above interpretation enabled to estimate the amount of physisorbed water present in the system (bound in the structure of hybrid gel) which is roughly the same in the whole range of gelatine/silica ratio and equals to about 7 – 9 %. It should be mentioned that the resistance of the hybrid system G1 to thermal decomposition of protein component (T_{max} = 670 K, 730 K) is considerably higher than other hybrids. As protein component content in the investigated materials increases, the maximum of thermal decomposition shifts to lower temperatures. The obtained hybrid systems are characterized by a higher resistance to protein component decomposition compared to systems prepared by other authors [23].

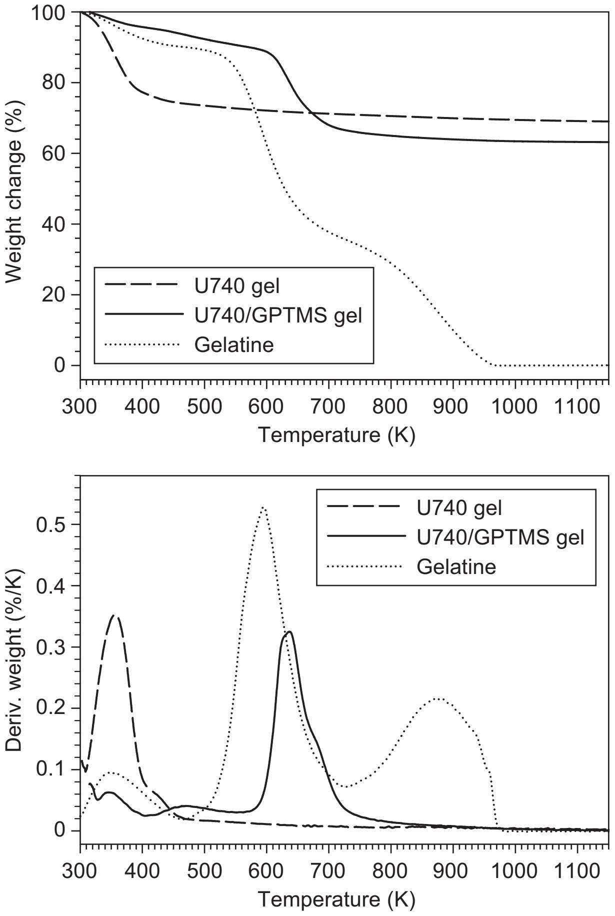


Figure 7. TG and DTG curves of reference samples (measurements carried out in air flow after gel drying at 293 K for 14 days).

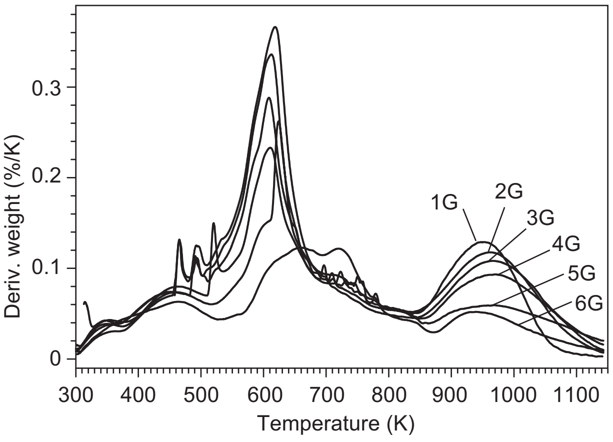


Figure 8. DTG curves of hybrid samples (measurements carried out in air flow after gel drying at 293 K for 14 days).

CONCLUSIONS

- Alternative method was developed for synthesis of gelatine/silica hybrid system with the use of functionalized silica sol. It differs from the method based on direct coupling of amino and carboxyl groups of protein using GPTMS and introducing such a system into silica sol. Moreover, another precursor of SiO_2, such as ethyl silicate, was employed, which was the likely reason for obtaining highly homogeneous structures.
- Thermal analysis data showed that the thermal decomposition of modified gelatine took place above 590 K. The thermal decomposition of the hybrid proceeded in two stages - at the first one the protein structure destruction and carbonization occurred, whereas at the next stage carbonaceous deposit was burned.

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