



Synthesis and Characterization of Gelatin-Siloxane Hybrids Derived through Sol-Gel Procedure

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Abstract. A new type of inorganic-organic hybrid materials incorporating gelatin and 3-(glycidoxypropyl) trimethoxysilane (GPSM) was prepared through sol-gel processing. A solid-state ^{29}Si NMR analysis indicated that all the methoxy silane groups of GPSM were polymerized to yield $-\text{Si}-\text{O}-\text{Si}-$ bridging bonds. An amino acid analysis confirmed grafting reactions of GPSM against gelatin chains. The increasing GPSM/gelatin ratio stimulated gel formation, phase separation, and the density of GPSM-crosslinking of the gelatin chains as well as it changed the micro- and macro- structures and the viscoelastic properties of the final products.

Keywords: sol-gel method, gelatin, siloxane, hybrid

1. Introduction

Bioactive ceramics include hydroxyapatite [1], β -tricalcium phosphate [2], and some silicate glasses and glass ceramics like Bioglass[®] [3] and A-W GC[®] [4], to name a few, that spontaneously form an apatite layer on their surface under body environment, the layer which can play a structural glue between the materials and the tissues [1, 3, 4]. Natural polymers like chitosan, chitin, or gelatin have already been employed in clinics by virtue of their bioresorption and flexibility [5, 6] though they are not bioactive. Hybrid materials derived from the integration of those polymers with some ceramics species may be both biodegradable and bioactive hence can construct a group of artificial materials appropriate for soft or hard tissue replacement. The hybridization may tame the brittle character of the bioceramics, and may even lead to ease of on-site modification of shape.

Sol-gel processing has been studied for attaining such synergetic effects of hybridization among inorganic and organic species [7–12]. Furthermore, a few research groups have demonstrated that they can hybridize inorganic Si–O or Ti–O groups into organic materials such as polydimethylsiloxane and polymethylmethacrylate, in order to provide them with

bioactivity through sol-gel processing [11, 12]. However, most of the research has been concerned with the hybrids of metal alkoxides and organosilanes that are not biodegradable. The use of natural polymers like proteins or polysaccharides as the organic components should lead to both bioactivity and biodegradability of the resultant hybrids. In the present work, gelatin-siloxane hybrids have been synthesized where 3-(glycidoxypropyl) trimethoxysilane (GPSM) is grafted to the gelatin chains at the epoxy end and the grafted GPSM molecules form cross-links through hydrolysis and condensation of the methoxy silane groups at the other end. The microstructure of the hybrids has been systematically examined and the effects of the composition are presented on morphology and crosslinking density. The bioactivity and biodegradability of the hybrids will be reported later.

2. Experimental

2.1. Preparation

All commercially available chemicals employed in the present sol-gel synthesis were of reagent grade and were used without further purification. Appropriate

amounts of GPSM were added to a 12.5 mass% gelatin solution in 0.1 M HCl under stirring at 40°C so that the mass fractions of GPSM (f_G) = 0.09, 0.23, 0.33, 0.50, 0.67, 0.75, and 0.91 were attained. Though the mixtures initially formed two-phase emulsions, continuous stirring gave clear homogeneous solutions. The precursor sols were further stirred for additional 30 min after homogeneity. Each 20 ml portion of the sols was poured into a polystyrene container 91 × 69 × 26.5 mm in size with a cap and aged for gelation at 40°C in an oven. Gelation time was defined as the duration of time until the precursor sols lost fluidity. The wet gels were dried at 60°C for 7 days. The appearance of all hybrids was listed in Table 1. Here, shrinkage was represented by the difference in surface area between the wet gels and the dry gels. The measurement was repeated three times for each sample to give standard deviation.

2.2. Characterization

The microstructure of the surface of the hybrids was examined with a scanning electron microscope (SEM, JEOL JSM-6300) at 20 kV acceleration after coating a 30 nm Au film. The solid-state ^{29}Si cross-polarization/magic-angle spinning (CP-MAS) NMR spectra (59.6 MHz) were taken using a Varian UNITY INOVA300 instrument with 3.5 kHz specimen spinning, 2.5 ms contact time, 5.0 μs pulse width, 10 s recycle delays and 10 μs dead time, where the signals from about 8000 pulses were accumulated. The chemical shift is represented in δ (ppm) by convention. Polydimethylsilane (PDMS: $\delta = -34.0$ ppm against tetramethylsilane: $\delta = 0$ ppm) was used as the secondary external reference. The dynamic mechanical properties were examined with a Seiko DMS120 dynamic viscoelastic ana-

lyzer for the hybrids with 20 mm in length at 0.5, 1, 2, 5, and 10 Hz as they were heated at the rate of 2°C/min.

Dry specimens absorbed water and swelled when soaked in distilled water. The swelling degree (S_w) was defined as: $S_w = (W_h - W_d) / W_d \times 100$ (%), where W_d was the dry weight, and W_h was the wet weight of a specimen dependent on the soaking time. The measurement was repeated five times to give the standard derivation.

Pure gelatin (15 mg) and the hybrids (15 mg) were hydrolyzed in 3 ml of 6 N HCl at 110°C for 22 h, and then the solutions were centrifuged for 5 min at 2900 r.p.m. The supernatant liquid was examined with an amino acid analyzer (Model ACL-1000, Shimadzu) equipped with a Li^+ type of column for quantitative analyses of the yielded amino acids. The quantity of the amino acids was normalized to that in the hybrids containing 100 g gelatin, and denoted by M_h and M_g for the hybrids and gelatin, respectively.

3. Results and Discussion

3.1. Microstructure Development

Table 1 demonstrates that f_G has a significant effect on the gelation of the gelatin-siloxane hybrids. Much lower or higher GPSM contents resulted in no gel formation, while moderate incorporation of GPSM into the gelatin solution favored gelation. Moreover, Table 1 shows greater shrinkage for larger GPSM contents. The morphology of the gels was also dependent on f_G . The hybrids with $f_G \leq 0.50$ were featureless, as represented in Fig. 1(a) for $f_G = 0.50$, indicating a homogeneous microstructure. Translucent and opaque appearance of the hybrids with $f_G \geq 0.67$ (Table 1) suggested they

Table 1. The preparation and characteristics of gelatin-siloxane hybrids and GPSM gel.

Hybrid (f_G^a)	GPSM/Gelatin (mass ratio)	Gel feature	Gelation time (hours)	Shrinkage (%)
0.09	0.1	Homogeneous sol	— ^b	— ^b
0.33	0.5	Yellow, transparent	120	1.8 ± 0.2
0.50	1	Yellow, transparent	72	9.0 ± 0.3
0.67	2	White, translucent	48	21.6 ± 1.8
0.75	3	White, translucent	24	39.1 ± 3.2
0.91	10	Heterogeneous sol	— ^b	— ^b
GPSM gel	— ^c	transparent	504	45.8 ± 2.1

^aWeight fraction of GPSM.

^bNo gel formation.

^c $f_G = 1.0$.

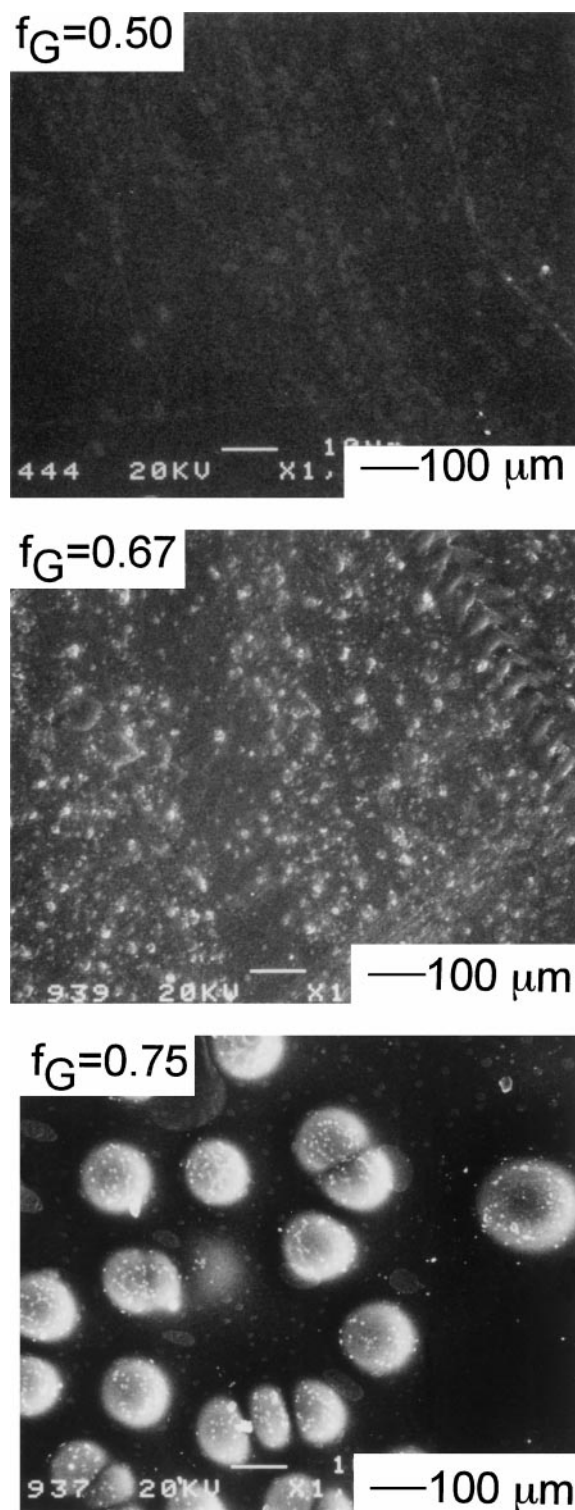


Figure 1. SEM photographs of gelatin-siloxane hybrids with $f_G = 0.50$, 0.67 , and 0.75 respectively. Hybrids with $f_G = 0.50$ were similar in the morphology to hybrid with $f_G = 0.50$.

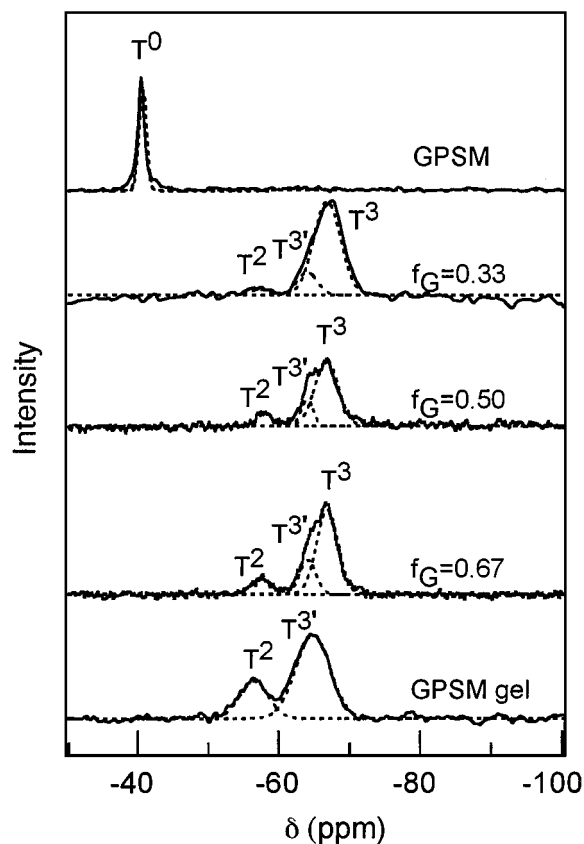


Figure 2. ^{29}Si CP-MAS NMR spectra of GPSM, GPSM gel, and gelatin-siloxane hybrid gels with $f_G = 0.33$, 0.50 , and 0.67 , respectively. Peaks T^2 , $T^{3'}$, and T^3 are assigned in the text.

were phase separated. Indeed, Fig. 1 (b) and (c) confirmed heterogeneity represented by the bright spots growing in size with f_G . From an EDX analysis, the bright spots were rich in Si. The phase separation in the present system is discussed later.

In the present solution system of GPSM— 0.1 M HCl —gelatin, one can expect that the methoxy silane groups ($\text{Si}-\text{OCH}_3$) of GPSM are hydrolyzed to give silanol ($\text{Si}-\text{OH}$) groups and result in $-\text{Si}-\text{O}-\text{Si}-$ bridging bonds [13]. This is confirmed with the ^{29}Si CP-MAS-NMR spectra in Fig. 2 for GPSM, the hybrids, and the GPSM gel. Here, the siloxane units are conventionally represented by T^n . Each T^n denotes a group $\text{R}-\text{Si}(-\text{OSi})_n(\text{OCH}_3, \text{OH})_{3-n}$ ($n \leq 3$) [14, 15], where R is the organic skeleton from GPSM. A peak at -40 ppm for monomeric GPSM was then denoted as unit T^0 . The GPSM gel from a 0.1 M HCl solution in molar ratio GPSM: $\text{H}_2\text{O} = 1 : 3$ gave no other peaks than at -56.3 and -64.1 ppm assigned to units T^2 and $T^{3'}$, respectively. This indicates all of the Si

atoms of GPSM are condensed to yield two or three bridging bonds. However, it is not certain only from the NMR data if all of the methoxy silane groups were hydrolyzed or remained. Figure 2 also indicates the growth of peak T^2 for the hybrids with increase in f_G . Moreover, one may observe a shift of peak T^3 toward high magnetic fields (toward more negative values in δ) with decrease in f_G . Careful inspection reveals that this superficial shift was caused by the growth of a shoulder peak (T^3) on the high field side of peak $T^{3'}$. With the resolution of the present NMR spectrometer it should be inappropriate to resolve peaks T^2 and T^3 into the component peaks corresponding to each $T^2(-T^3)_x(T^2)_{2-x}$ or $T^3(-T^3)_x(T^2)_{3-x}$ ($X = 1, 2$ or 3). Therefore, the NMR spectra of the hybrids may be resolved with one T^2 peak and two T^3 peaks because of less skewness of peak T^2 and of the presence of the shoulder for the T^3 peak. Relative abundance of each T^n unit has then been derived from the deconvolution of the peaks with three Gaussian functions and listed in Table 2. It is indicated that the fraction of T^2 increased with f_G at the expense of that of T^3 and $T^{3'}$. The fact that peak T^3 appears in the high field side of peak $T^{3'}$ derives that units T^3 bond to a less number of units T^2 than units $T^{3'}$. Thus, the number of $-\text{Si}-\text{O}-\text{Si}-$ bridging bonds are expected to decrease with gelatin.

Under acidic conditions, GPSM is subject to cleavage of the C—O bond in the epoxy group [16]. The protonated epoxy group is so activated to attack nucleophilic groups like $-\text{NH}_2$, or $-\text{COOH}$ on the amino acid residues comprising the gelatin chains that GPSM molecules are grafted to the gelatin chains. It is thus likely that the hydrolysis and condensation of the metal alkoxides and ring-opening reactions of the epoxy groups yield a crosslinked network in the gelatin-siloxane hybrids as illustrated in Fig. 3 (Gelatin skeleton) $-\text{R}-\text{Si}-\text{O}-\text{Si}-\text{R}-$ (Gelatin skeleton) (R denotes organic skeleton of GPSM) bonds are formed

Table 2. Chemical shift (δ) and fraction (%) of T^n siloxane structures in gelled materials.

Sample	T^2		$T^{3'}$		T^3	
	δ (ppm)	% ^a	δ (ppm)	% ^a	δ (ppm)	% ^a
GPSM gel	-56.3	27.1	-64.1	72.9	ND ^b	
Hybrid: $f_G = 0.67$	-57.4	12.1	-63.8	20.7	-66.5	67.2
Hybrid: $f_G = 0.50$	-57.7	9.8	-63.6	15.2	-66.4	75.0
Hybrid: $f_G = 0.33$	-57.8	3.4	-63.5	15.0	-67.0	81.6

^aThe fraction in peak area.

^bNot detected.

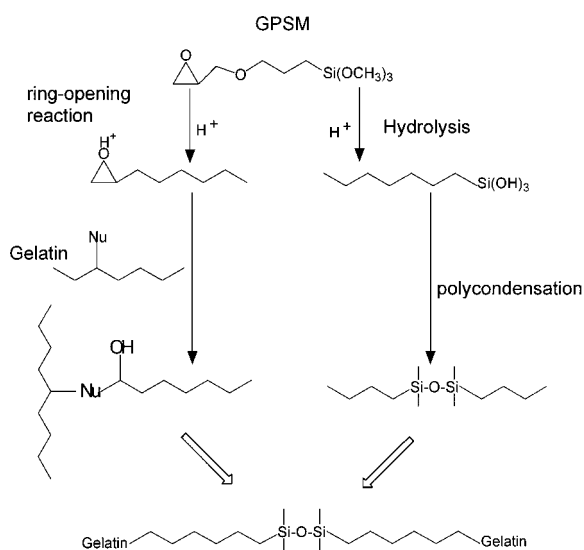


Figure 3. Gelatin-siloxane hybrids are suggested to be derived from the hydrolysis and polycondensation of GPSM with water and the ring-opening reaction of GPSM with gelatin.

as the GPSM molecules bridge a fraction of such nucleophilic active sites on the side groups of the amino acid residues. ^{13}C -NMR spectroscopic analysis could not add definite information on the interaction between the epoxy group of GPSM and gelatin molecules due probably to the complex structure of gelatin. The fraction of the amino acid residues grafted with such bridging bonds can be evaluated in the following way. Though hydrolysis fully dissociates and breaks the gelatin chains into the component amino acids, the components bonding to some grafting molecules will not be hydrolyzed to the corresponding amino acid residues but remain as GPSM-amino acid fragments due to stronger and stabler grafting bonds. Let Z represents the ratio M_h/M_g , so that Z should indicate the fraction of the amino acids released at hydrolysis out of the present gelatin component in the gelatin-siloxane hybrid. Therefore, $1 - Z$ listed in Table 3 represents the fraction of the amino acid residues that were not dissociated in the hydrolyzing solution. In other words, $1 - Z$ corresponds to the fraction of the amino acid residue involved in the crosslinking bridges. With the least f_G , Arg, Asp, Thr, and Ile were fully immobilized, that is, they had the strongest susceptibility for GPSM grafting. This is attributed to the fact that they involve nucleophilic groups. As f_G increased Glu, Hyl, Lys, Ser, and Val were the next to be immobilized. The latter 5 groups were much insusceptible to the grafting attack of GPSM. With the largest content of GPSM

Table 3. The fraction of amino acid residues involved in the crosslinking bridges as determined by amino acid analysis.

Amino acids	1-Z (%)		
	$f_G = 0.33$	$f_G = 0.50$	$f_G = 0.67$
Ala	11.2	11.2	14.3
Arg	100.0	100.0	100.0
Asp	100.0	100.0	100.0
Glu	30.2	100.0	100.0
Gly	5.3	4.3	5.3
Pro	12.1	50.3	100.0
Hyl	18.9	100.0	100.0
Ile	100.0	100.0	100.0
Leu	10.3	28.2	23.1
Lys	54.2	100.0	100.0
Phe	32.2	21.4	100.0
Ser	36.6	100.0	100.0
Thr	100.0	100.0	100.0
Val	15.7	100.0	100.0
R_{graft}^a	0.99	0.95	0.56

^aThe molar fraction of GPSM involved in the grafting.

some fractions of Ala, Gly, and Leu still remained. In this respect, it is concluded that Arg, Asp, Thr and Ile are the most favorable bridging sites on the gelatin skeletons. The conclusion is accounted for by the extent of electrophilic activity of each amino acid residue. The molar fraction of GPSM involved in the grafting (R_{graft}) was derived from the hydrolysis data and represented in Table 3. Note that R_{graft} for the hybrids with f_G of 0.33 and 0.50 was almost equal to 1, while R_{graft} for f_G of 0.67 was only 0.56. This indicates that as the GPSM content exceeds a threshold value, the epoxy group of GPSM cannot find the active sites of the gelatin chains since most of the active $-\text{NH}_2$ or $-\text{COOH}$ groups are already attacked to form gelatin-GPSM graft bonding.

3.2. Phase Separation

SEM photographs in Fig. 1 have confirmed opacity and transparency for the hybrids with $f_G \geq 0.67$ shown in Table 1. Thus, it is concluded that an excess amount of GPSM over a threshold in the hybrids is assembled together to form clusters to be phase separated. The presence of a larger fraction of unit T^3 bonding to more units T^2 superficially seems favorable for the segregation of the siloxane units since it should provide a greater number of Si—O—Si bridging bonds. However,

the experiments have exhibited the contrary. The ^{29}Si NMR spectrum for the hybrid with $f_G = 0.67$ in Fig. 2 indicates a stronger lower-field component (T^3) as well as a stronger peak T^2 , both of which are the primary units of the GPSM gel. It is thus derived the segregation requires more units T^2 . This can be interpreted as indicating that increase in unit T^3 results in the growth of sheet-type structures like that of disilicate ions $\text{Si}_2\text{O}_5^{2-}$ [17] and leads to lose flexibility and stability of the Si—O—Si skeleton in the GPSM gel while the increase in T^2 introduces a chain structure similar to metasilicate ions SiO_3^{2-} [17] to attain flexibility and stability, which favors clustering. It is natural that the number of such $-\text{Si}-\text{O}-\text{Si}-$ bonds increases with the increase in the GPSM content as gelation involves condensation of the Si—OH groups. Hydrophobic character of the gelatin chains may also contribute to phase separation when one considers that the gelatin chains are basically hydrophobic or covalent in character though it consists of peptide bonds and has a few kinds of hydrophilic groups like $-\text{NH}_2$ and $-\text{COOH}$ chemically compatible with such siloxane groups.

3.3. Density of Crosslinking

Table 3 has indicated that the hybrid with the least amount of GPSM (0.33 in f_G) involved a much less fraction of the amino acid residues for the crosslinking bridges than the other hybrids. The difference in the crosslinking degree among the hybrids will be reflected on the swelling property and dynamic mechanical/thermal properties. The weight loss for the hybrids in distilled water was negligible small ($<1\%$) in the shorter time (<2 days), and it thus was neglected at deriving swelling degree. Gelatin absorbs water to swell up or even is dissolved away when soaked in water [6]. The introduction of GPSM may restrain the gelatin component in the hybrids from swelling because of tightening effects of the bridging bonds. Figure 4(a) shows the weight gain per unit weight of the hybrids or the increase in volume ΔV at absorbing water into the hybrids as a function of the soaking time. It is indicated that the hybrids absorbed water quickly within a few hours of soaking and ΔV reached a maximum (ΔV_{max}) characteristic of each hybrid. Gelatin and the hybrids with $f_G < 0.33$ were dissolved into water in soaking for 1 h, while the hybrids with $f_G \geq 0.50$ were only found to swell at soaking up to 48 h. With very small ΔV_{max} for the GPSM gel, one should conclude that the gelatin component is responsible for swelling. Thus

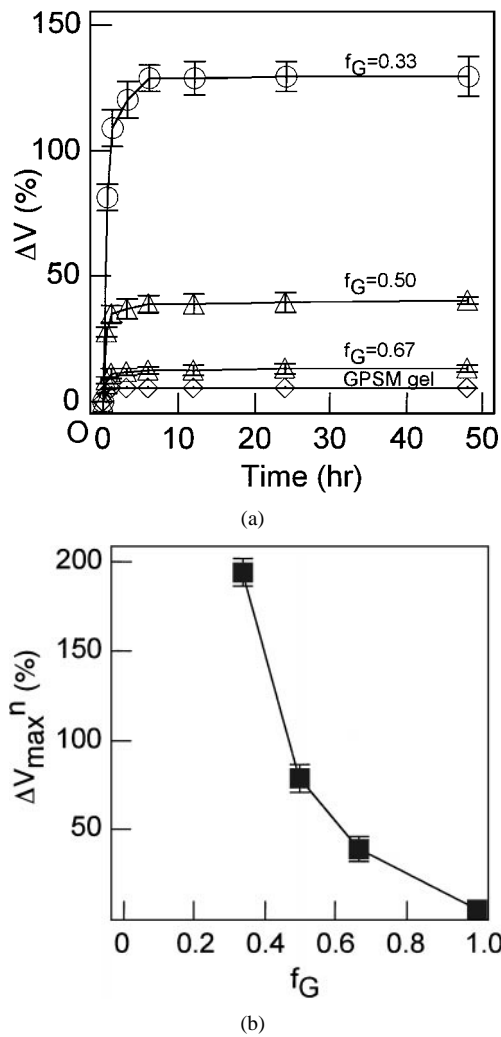


Figure 4. (a) The increase in volume (ΔV) at absorbing water into the gelatin-siloxane hybrids as a function of the soaking period. Gelatin and the hybrids with $f_G < 0.33$ were dissolved into water on soaking for 1 h, while GPSM gel swelled little at soaking up to 48 h. (b) The maximum of swelling degree ΔV_{\max}^n for the gelatin-siloxane hybrids in distilled water up to 2 days as a function of the weight fraction of GPSM. The change slope at $f_G = 0.50$ suggest a structural change.

ΔV_{\max} is related to the ability of the gelatin component to absorb water. In this respect, ΔV_{\max} has been converted to that (normalized $\Delta V_{\max}^n = \Delta V_{\max}^n / 100$) of the hybrids containing 100 g-gelatin and plotted in Fig. 4(b) as a function of f_G . The slope of the $\Delta V_{\max}^n - f_G$ curve corresponds to the volume decrease around the gelatin component, i.e., decrease in the free volume admitting water associated with a unit weight of gelatin. The steeper slope in the range $f_G \leq 0.50$ and sudden decrease in slope at 0.50 suggest a structural change. With

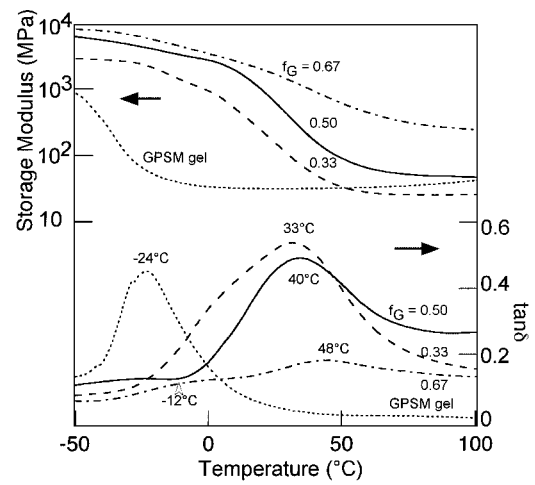


Figure 5. Storage modulus (E') and $\tan \delta$ of the gelatin-siloxane hybrids and GPSM gel as a function of temperature.

the observation that R_{graft} in Table 3 for the hybrid with $f_G = 0.67$ is only 0.56, one can conclude from Fig. 4(b) that the crosslinking degree of the gelatin chains in the hybrids with $f_G \geq 0.50$ is much higher than the hybrids with $f_G < 0.50$, and that $f_G = 0.50$ is associated to the limit of homogeneous distribution of GPSM without phase separation.

The structural model deduced above can account for the mechanical or viscoelastic properties of the hybrids in Fig. 5 where dynamic mechanical storage modulus (E') and $\tan \delta$ are plotted as a function of temperature. It is reasonable that the hybrid with the largest f_G gave the largest E' . Glass transition temperature (T_g) represented by a peak temperature for a $\tan \delta$ -temperature curve is a qualitative measure of the ease of lattice motion of the constituents. Moreover, the temperature, intensity, and width of a $\tan \delta$ band may represent the ease of relaxation movement, the quantity, and broadness of dispersion of the species, respectively [18]. Since GPSM gel showed no peak in the range $> 30^\circ\text{C}$, the peak of $\tan \delta$ -temperature profiles was attributed to the gelatin chain. The increase in peak temperature, T_g , from 33°C to 48°C with increase in f_G is attributable to the segmental movement of the gelatin chains restrained by the bridging or crosslinking bonds involving the GPSM molecules. This agrees with the interpretation on the increase in apparent activation energy of the T_g peak listed in Table 4. Moreover, the decrease in the T_g peak intensity corresponds to the decrease in the quantity of the gelatin chains resonating at the temperature. This suggests the size of network mesh decreases because of the increased density of crosslinking

Table 4. Viscoelastic properties of hybrids and GPSM gel.

Sample	E' (36.5°C) (MPa)	T_g (°C)	ΔH^{*a} (KJ/mol)
Hybrid: $f_G = 0.33$	73	33	175
Hybrid: $f_G = 0.50$	196	40	250
Hybrid: $f_G = 0.67$	985	48	456
GPSM gel	21	-24	91

^a Apparent activation energy.

and the longer segments of the gelatin chain responsible for the high temperature peak decrease in number. A small shoulder was observed on the low temperature side: at 0°C for the hybrids with $f_G = 0.33$ and 0.50, and at -12°C for $f_G = 0.67$. Since the hybrid with $f_G = 0.67$ was phase separated, we believe the peak of -12°C was due to the movement of -Si-O-Si- oligomers in accord with that of GPSM skeletons like that for the -24°C peak of the GPSM gel [19]. The decrease in temperature of the shoulder peak with f_G was due probably to increased condensation of the siloxane units.

4. Conclusions

In the present study, a new family of organic-inorganic hybrid materials, which incorporated gelatin chains covalently into siloxane network was synthesized through sol-gel procedure. ²⁹Si CP-MAS-NMR spectra indicated that methoxy silane groups of GPSM were hydrolyzed and resulted in -Si-O-Si- bridging bonds; while amino acid analysis demonstrated that the GPSM molecules were grafted to the gelatin chains due to the reactions between the epoxy groups of GPSM and the nucleophilic active groups of gelatin. Thus, this process provides inorganic-organic hybrids in which the inorganic component of Si chemically bonded to natural

polymer of gelatin with -Si-O-Si- bonds. The gel forming, the macro- and micro-structure, the phase separation, and the crosslinking density of these hybrids depend on their molecular composition and can be tailored by varying the fraction of GPSM.

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