Structure and Relaxation Properties of Medical-Purposed Polyacrylamide Gels

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Received 9 January 2004; accepted 1 July 2004
DOI 10.1002/app.21477
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: By IR and NMR spectroscopy methods, thermomechanical analysis, mechanical relaxation measurements, and computational simulation the effect of production conditions of acrylamide copolymer and *N,N'*-methylenebisacrylamide derived hydrogels with respect to their properties were studied. Four hydrogel samples, prepared under different production conditions (γ-radiation dose and autoclaving), were investigated. It was found that autoclaving and γ-radiation lead to a slight increase of the crosslinking degree in the polymeric network and formation of alkene structures in polymeric chains. Stress relaxation and creep processes under axial compression of gel cylindrical samples were studied in detail. To approximate stress relaxation and creep curves new memory functions were used, based on the analysis of entropy production in the system during the relaxation process. It was found that primary γ-radiation of initial gels induces an increase of quasi-equilibrium rubbery elasticity modulus, and quasi-equilibrium compliance is decreased. The opposite situation is observed during further autoclaving at 120°C. After autoclaving, required to sterilize the gels, their treatment by γ-radiation again induced a noticeable increase of the modulus and compliance decrease. The mechanism of relaxation processes was found to be associated with the limiting stage of physical interaction between relaxants, representing different micro-inhomogeneities in the material. The investigation results were compared with the data obtained by histology and morphology methods. A hydrogel obtained under additional γ-radiation treatment and autoclaving did not swell when implanted into a living organism, and the tissue reaction to implantation of such gel was minimal. J Appl Polym Sci 96: 1043–1058, 2005

Key words: stress; relaxation; γ-radiation; crosslinking; creep

INTRODUCTION

Polymeric hydrogels derived from polyacrylamide, crosslinked by a small amount of a bifunctional compound, have found wide applications in the fields of agriculture and medicine.[1–10] The water content for production of such gels reaches 95–97%. Because hydrogels are characterized by a system that consists of a polymer network with a large amount of water, different methods are used to study the gel structure, which is why the gel structure is determined by both the polymer network structure (crosslinking density, chemical structure of crosslinking agent, crosslink distribution, various defects of the network, etc.) and parameters of the macrostructure (porosity, pore distribution by sizes, crystallinity, water conditions in the system, etc.).

Parameters such as crosslinking density, average molecular mass of linear fragments between crosslinked points of the network, and chain concentration in the specific volume are determined from measurements of the equilibrium rubbery modulus[11–19] and—which is the most suitable for the case of hydrogels—by the degree of equilibrium swelling.[1,20,21] Results from studies of elastic properties obtained for gels by dynamic light scattering method are also used.[22]

Among water-containing polymeric gels, hydrogels derived from polyacrylamide are among the most widely investigated. These gels are derived from the product of acrylamide copolymerization with bifunctional compounds, low amounts of which are injected into the reaction mixture for the purpose of forming a polymer network. Concerning the initial linear water-soluble polyacrylamide, it is representative of the class of polymers whose properties have been studied in the most detail. The features of synthesis of this polymer, its structure, and properties are described in detail in a series of monographs and reviews, among which are the notable studies reported by Gromov and Teleshov,[23] Sinani,[24] Chupov,[25] Abramova et al.,[26] Serovitskaya and Khomdova,[27] and Plate and Vasil'ev.[28] There are many works in which polyacrylamide and gels derived from it are synthesized by γ-radiation.[29–37]

Reactions of polyacrylamide copolymerization with bifunctional compounds, such as *N,N'*-methylenebi-

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sacrylamide (MBAA) and *N,N′*-ethylenebisacrylamide (EBAA), for example, have been among those most thoroughly studied. Among the works in which these reactions have been investigated in the greatest detail, the studies by Tajuddin and Anwaruddin[38] and Ilavsky et al.[39] deserve special mention. Studies of mechanical properties of hydrogels derived from these copolymers indicate the best properties of acrylamide (AA) copolymers with MBAA. Naturally, mechanical properties depend on both the crosslinking density of the copolymer matrix (i.e., on the AA : crosslinking agent ratio) and the amount of water in the gel.[40–43] Thus, gels containing a large amount of MBAA are high-modular compounds possessing high friability.

Recently, it has been accepted that gel from polyacrylamide is not homogeneous but rather possesses, at least, a two-phase structure.[44–47] At the initial stage of acrylamide copolymerization with MBAA, *N,N′*-methylenebisacrylamide microgel particles are formed with chemical bonds between the chains located inside of the particle. At the second stage, these microgel particles form infrequent crosslinks between one another, leading to gel formation.[48,49] It should be noted that at the initial stage a defective network, with anomalously added units (for example, head-to-head) and different cycles and dangled chains, is formed.[50–55] Therefore, these suspended chains can also represent MBAA residues, joined with one end in the linear chain. Further on, at the later stages of the reaction these dangled chains can form additional infrequent crosslinks. Dimensions of zones with more frequent networks, determined by small-angle neutron scattering and quasi-elastic light-scattering methods,[56,57] are estimated up to 500 nm. Such a heterophase structure of polyacrylamide hydrogels imparts their high permeability and high mechanical properties. (These problems are studied in detail in works by Baselga et al.[15] and Nossal.[22]) The highest elasticity modulus (0.311 N/mm^2) is typical of the networks, synthesized in high concentrations of monomers (18 g/100 mL) and the crosslinking agent content of 10.1%. At this content of the crosslinking agent the modulus is abruptly decreased with total concentration of monomers. This is explained by formation of gels containing a great quantity of microgel particles, weakly crosslinked with one another, in the diluted system. At the same time, the presence of such particles induces a reinforcement that promotes the high strength of gels.

Note also that described in the cited literature are mainly border mechanical properties of hydrogels, such as nonequilibrium elasticity modulus, deformability, and strength. Scant attention was devoted to the relaxation properties of hydrogels derived from polyacrylamide. Meanwhile, it is quite obvious that gels under stresses and deformations approaching destructive limits will not be used. Because gels are used under stresses and deformations below the border values, the relaxation processes—stress relaxation and creep—are developed in them. Of special importance is estimation of the extent of relaxation processes for medical-purposed gels. This problem will be discussed in more detail.

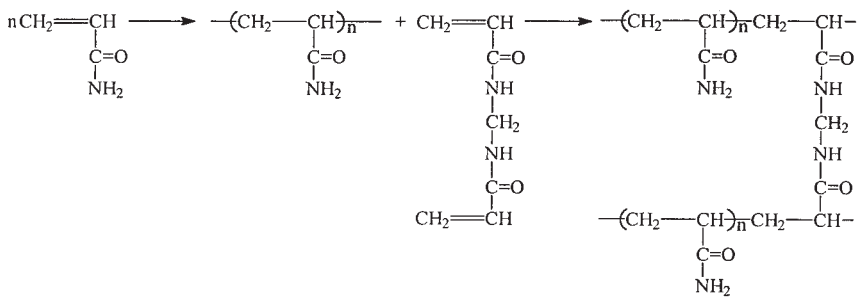
Presently, water-swelling network polymers derived from polyacrylamide are widely used in decorative surgery as injective implants for facial and soft tissue plastics.[58–64] The advantage of these copolymers over other injective materials is the low concentration of polymer in the hydrogel (3–4%). Thus, the amount of gel injected into the organism can be increased to 200–300 g.

However, there are definite problems in deriving medical gels from polyacrylamides. Even a small change in radical polymerization parameters during synthesis of such materials, or during their future processing, can cause a significant change in the gel molecular network and, consequently, a change of its medical–biological properties.

The effect of synthesis conditions and monomer composition in the initial mixture on the swelling degree and the average distance between crosslinked points of the network was previously studied.[65,66] The results of histological and morphological studies with respect to the effect of various polyacrylamide gels on tissue reaction—which is the indispensable characteristic for use in medical materials—are presented in the works by Lukomsky et al.[60] and Shekhter et al.[62] Qualitative assessment of the effect of some functional groups on *in vivo* behavior of hydrogels was also performed.

However, publications devoted to complex systematic investigations, which would allow determination of the effect of structural features of the gel molecular network and its various structural fragments on the most important properties of medical polyacrylamide gels, especially on biological, morphological, and histological characteristics, have not been produced. Further investigations in this field are of paramount importance because the structure of the material produced from gels is quite specific. It is formed not only during synthesis of the polymer, but is also changed at different stages of its future processing, required for medical application. Finally, after creation of the yield form of the material—for example, a syringe for injection—the gel structure must be fixed, and the process of further possible formation of metabolites must be strictly defined. Thus at all stages of gel production and processing its structure must be regulated to provide for safety and reliability of application of the ready medical materials.

Because several specific requirements are imposed on modern injection materials for medical purposes, such as intact property, biocompatibility, and noninvasiveness at injection, it seemed necessary to study the effect of the basic parameters of the gel production



Scheme 1

not only on its structure, but especially on the tissue reaction after gel injection into the living organism. At the contour plastics of soft tissues and facial plastics hydrogels are injected using thin needles, which prevent traumatic aftereffects of the procedure: that is why hydrogels must possess high plasticity. During this type of injection, the gel is mechanically stressed and, as a result, creep is developed, or it is deformed, which induces stress relaxation. On the other hand, during implantation of gels to the organism it is important to prevent rampant progression of the processes, because after injection the gel must maintain its shape for a long time and not migrate with time, affected by muscles. That is why the study of stress relaxation and creep processes of hydrogels, applied in medicine, in particular, is the actual problem. In the current work the study is performed for hydrogels derived from crosslinked polyacrylamide, prepared under different processing modes, with respect to gel production and sterilization processes.

TEST SAMPLES

Test samples were prepared as follows.

Sample 1. This sample was prepared with a mixture of (in g): acrylamide (AA), 3.8; *N,N′*-methylenebisacrylamide (MBAA), 0.032; and ammonium persulfate, 0.054. All products used were obtained from Aldrich Co. (Milwaukee, WI). The prepared sample was dissolved in 107 mL of distilled water at 40–50°C in the following sequence: AA first, then MBAA, and finally ammonium persulfate. After that the solution was filtered in a Büchner funnel through a polymeric filter (pore size 45 μm). The filtered solution was poured into flasks of 20 mL capacity each by 10 mm height. The flasks were sealed by rubber stoppers and seamed by tin lids. Then they were placed into an air thermostat and exposed at 70 $\pm$ 4°C up to gel formation (for 2 h). When thermostatic control was completed, the sample gel in the flasks was radiated on γ-unit "Investigator." The radiation dose was 0.5 mrad.

Sample 2. It was prepared analogously to sample 1. After preparation, the flasks were opened, and the gel was washed by distilled water. The flasks were filled with water and exposed for 10 min, after which the water was poured off. Volumetric water : gel ratio was 1 : 4. The gel was washed twice. After that the flasks were sealed and radiated on the γ-unit by 0.5 mrad dose (for sample 2, total radiation dose was 1 mrad).

Sample 3. It was prepared similarly to sample 2, but after the radiation treatment the flasks were sterilized at 120°C for 40 min pressurized at 1.2 atm.

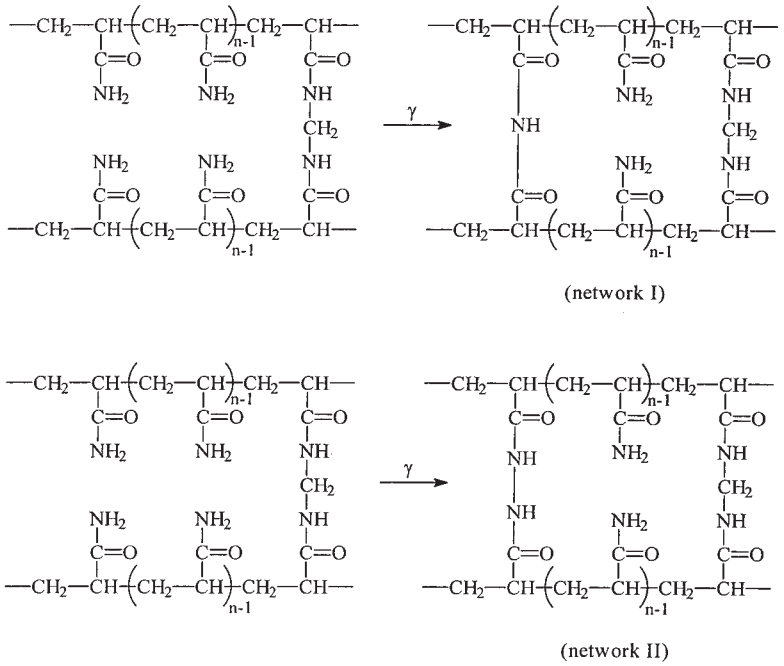
Sample 4. It was prepared similarly to sample 3. After sterilization, the gel was additionally radiated on the γ-unit by 1 mrad dose (for sample 4, total radiation dose was 2 mrad).

Sample 1 was prepared at the first stage of the process, during which AA and MBAA copolymerization proceeds at temperature below 90°C in water in Scheme 1. Provisionally, it should be noted that a histological study of sample 1 indicates aseptic inflammation of the tissue.

Sample 2 was obtained by γ-radiation of sample 1. Thus, the structure of the polymer obtained is apparently formed according to Scheme 2.

This suggestion is based on data from the literature,[67,68] where structuring of γ-radiated polyacrylamides is analyzed. Formation of imide groups is also described in the literature[69,70]; therefore, the imide groups can both participate in the crosslink structure and form cycles.[70] By studying the histology of sample 2 the authors found that the gel does not cause aseptic inflammation; the tissue reaction is expressed at a low level. After 2 months a thin capsule of connective tissue is formed around the gel. Further on, this capsule does not regenerate into fibrotic fragments that usually occur under application of different polymers.

Sample 3 was prepared by sterilization of the initial copolymer for 40 min at 120°C under 1.2 atm pressure. Thus, the pH of the gel was shifted toward alkaline from pH 3.5–4 to pH 5–8 with respect to duration of autoclaving. The smell of ammonia is thus noticed in the vessel and the gel becomes less dense (thins down). Possibly, the processes that occur during autoclaving proceed according to the following scheme: As studied in animals, the processed gel swelled rapidly and resorbed. That is why to confer higher density and lower swelling property to the gel, sample 4



Scheme 2

was produced by additional γ-radiation. The gel then became denser and swelled poorly in water. When tested in animals it gave a very good tissue reaction. Autoclaving is necessary for sterilization of gel.

It follows from the above that procedures usually used for sterilization of medical materials cause different effects on variation of the structure and properties of polyacryamide gels. It seemed interesting to perform analysis of chemical transformations of the hydrogel network polymer derived from the AA/MBAA copolymer at different stages of their current and future processing. In the present work, such analysis was performed by different methods.

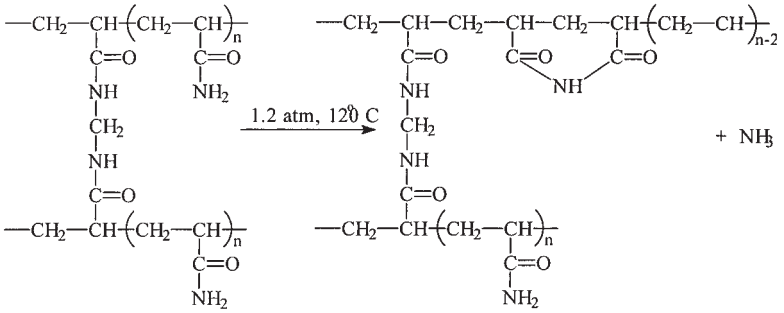
EXPERIMENTAL

IR spectra were obtained on a Magna-750 IR Fourier spectrometer (Nicolet Analytical Instruments, Madison, WI) with a spectral resolution of 2 cm^{-1}. The potassium bromide spectrum was subtracted from the spectrum of tablets using OMNIC software (Nicolet).

^{1}H- and ^{13}C-NMR spectra were recorded on an AMX 400 spectrometer (Bruker, Darmstadt, Germany). The operating frequencies were 400.13 and 100.61 MHz, respectively.

Thermomechanical analysis was performed under conditions of punch (of 4 mm diameter) penetration, loaded by 100 g weight, at a rate of temperature increase of 2°/min. As a result, temperature dependencies of deformation were determined.

Creep and stress relaxation tests were performed as follows. The creep of gel samples was measured on a modified Kargin weighing machine by the axial compression method. An analytical balance was used, to one beam of which the punch representing and flat plate was fixed. As 2 g weight was removed from the



Structure III

Scheme 3

balance pan, the plate transferred load to the sample. The samples were prepared as tablets (20 mm in diameter and 8 mm high). Deformation was measured with 0.001 cm accuracy that gave 0.125% relative deformation for the sample 0.8 cm high. Tests were performed over a period of 3 h, and deformation was recorded automatically.

Stress relaxation was measured on the Polyanyi-type dynamometer, under axial compression at constant deformation of 4.7% for all samples. This "immediate" deformation was set at a rate of 0.0075 mm/s. To avoid sample adhesion, the measuring surfaces were covered with an organosilicon liquid for both stress relaxation and creep measurements.

CALCULATION TECHNIQUE FOR GEL RELAXATION PARAMETERS

Parameters of materials derived on hydrogels were calculated by data of stress relaxation and creep tests using the Boltzmann–Volterra theory of viscoelasticity. By the present time, to describe stress relaxation and creep processes, different alternatives of memory functions in the Boltzmann–Volterra equation are suggested.[71,72] The memory functions possess three or four parameters and, usually, display a fractional time exponent because in this case only the experimental data on stress relaxation and creep can be described with good approximation.

Analysis of existing memory functions shows that, although at adequate selection of parameters they display progress of relaxation processes with sufficient accuracy, but the physical meaning of memory function parameters is not displayed. In this connection, an approach to obtain memory function, based on consideration of thermodynamic functions and their changes during relaxation process, is suggested.[18,19,73]

The main assumption suggested in the literature[18,19,73] is that stress relaxation or creep process proceeds as a result of interaction and diffusion of kinetic units, the relaxants. The relaxants can be various groups of atoms, repeating units, larger fragments of macromolecules, and their segments. Separate elements of free (in the present case, "empty") volume (i.e., macrocavities, stress concentrators, etc.) are relaxants also. Interacting with one another, these microcavities are able to fuse, restructure, and diffuse in the polymeric material during relaxation or creep, forming a structure that promotes a decrease of relaxing stress. The process transforming the initial microporous structure into a new balanced structure during stress relaxation is studied in detail, by the positron annihilation method, in works by Askadskii et al.[74,75] Actually, after unbalancing the initial microporous structure induced by "immediate" setting of deformation, a new microporous structure is formed during stress relaxation, which is well detected in the working cell of a spectrometer equipped with a device that measures stress relaxation.

If the above-mentioned is true, the polymeric material can be considered to consist of relaxants and nonrelaxants; the largest portion of the material after "immediate" setting of deformation consisted of relaxants interacting with one another and forming a nonrelaxing material. Occurrence of kinetic elements of two kinds (relaxants and nonrelaxants) and their diffusion in the material leads to production of entropy in the system, which is increased during stress relaxation.

Production of entropy (or the rate of entropy occurrence) is determined from the following expression: $(dS/dt)(1/v)$, where S is entropy, t is time, and v is the system volume. Memory functions were obtained[18,19,73] under the assumption that the motivating force of stress relaxation is entropy production in the system (sample), which increases during stress relaxation up to a maximal value. Considering entropy of mixing of two kinds of kinetic units (relaxants and nonrelaxants) we obtain

$$S = k_B \ln \frac{m^*!}{(\alpha m^*)![(1-\alpha)m^*]!} \tag{1}$$

where k_B is the Boltzmann constant, m^* is the total amount of kinetic units (in the present case, relaxants and nonrelaxants in specific volume), and α is the part of relaxants in the total amount of kinetic units.

On the basis of eq. (1), the following expression is obtained:

$$S = -k_B m^*[\alpha \ln \alpha + (1-\alpha)\ln(1-\alpha)] \tag{2}$$

The value of α changes with time from 1 to 0.5 because at $\alpha = 0.5$ the mix entropy becomes maximal.

It is assumed[73] that the memory function (in the Boltzmann–Volterra equation) is bound to entropy by reverse dependency as follows:

$$T(\tau) = S_0\left(\frac{1}{S} - \frac{1}{S_{\max}}\right)\int_0^\infty T^*(\tau)\, d\tau \tag{3}$$

where $T^*(\tau)$ is the variable part of the memory function.

By substituting eq. (2) into eq. (3) the following is obtained:

$$T(\tau) = -\frac{S_0}{k_B m} \times \left[\frac{1}{\alpha \ln \alpha + (1-\alpha)\ln(1-\alpha)} - \frac{1}{\ln 0.5}\right] \tag{4}$$

where $m = m^* \int_0^\infty T^*(\tau)\, d\tau$.

For the purpose of describing dependency of α on τ (recall that α is the part of relaxants in the total amount of kinetic units in the system), possible mechanisms of the relaxation process will be discussed. As mentioned earlier, variation of α with time τ can be explained by two reasons: interaction between relaxants and their transformation to nonrelaxants, and diffusion of kinetic units. Let us briefly consider these reasons and their consequences.

Because the interaction between relaxants is a complex process, it can be naturally described by an equation of the order n reaction. For example, if the order $n = 3$ is infrequently observed in a usual chemical reaction (because this requires active collision of three molecules at a time), then in the present case relaxants are accumulated in the sample and the elementary act of their physical interaction may simultaneously involve several relaxants (for example, fusion of several microcavities into a single one). Thus, the reaction order can be fractional. For this case, the actual kinetic equation is as follows:

$$\frac{dc}{d\tau} = kc^n \tag{5}$$

where k is the rate constant of the reaction and c is the concentration.

By integrating eq. (5) from $\tau = 0$ to τ, the following is obtained:

$$\mathrm{C} = \frac{\mathrm{C}_0}{[1 + \mathrm{C}_0^{n-1}(n-1)k\tau]^{(1/n-1)}} \tag{6}$$

where C_0 is the initial concentration of relaxants of any type (for the sake of simplicity it is accepted that these concentrations are equal for different types of relaxants). Then

$$\alpha = \frac{\mathrm{C}}{\mathrm{C}_0} = \frac{1}{(1 + \mathrm{k}^*\tau/\beta)^\beta} \tag{7}$$

where $k^* = k^{n-1}$, $\beta = 1/(n-1)$, and n is the reaction order.

Let us now consider the diffusion mechanism of relaxation. At random excitation of kinetic units the amount of places that the units occupy in the lattice at the moment of time τ and, consequently, the part of nonrelaxants $(1 - \alpha)$ is determined from the following expression:

$$(1 - \alpha) = at^{b/2} \tag{8}$$

where $0 < b < 1$ and a is a constant.

For $b = 1$ eq. (8) corresponds to Fickian diffusion, as follows:

$$1 - \alpha = \frac{4}{l}\left(\frac{D\tau}{\pi}\right)^{1/2} \tag{9}$$

where l is the sample size and D is the diffusion coefficient.

By substituting eq. (8) into eq. (4) we obtain

$$T(\tau) = -\frac{\mathrm{S}_0}{k_B m_2} \times \left[\frac{1}{\alpha\tau^\gamma \ln \alpha\tau^\gamma + (1 - \alpha\tau^\gamma)\ln(1 - \alpha\tau^\gamma)} - \frac{1}{\ln 0.5}\right] \tag{10}$$

where $\gamma = a/2$.

The function $T_2(\tau)$ is physically meaningful only when $a\tau^\gamma \leq 0.5$. The memory function (10) contains three parameters: $A = S_0/k_B m_2$, a, and γ. At $\tau = 0$ it represents the function with a weak singularity.

Memory functions (10) and (4) allow description of stress relaxation and creep processes with high accuracy, as well as estimation of physical parameters of the material: $A = m^*/S_0$ proportional to the amount of inhomogeneities in the material; k^*, n, γ, a, σ_0 (E_0), σ_∞ (E_∞), ε_0 (J_0), ε_∞ (J_∞), where σ_0 is the initial stress occurring after "immediate" setting of deformation; E_0 is the immediate elasticity modulus; σ_∞ is the equilibrium stress set at $t \to \infty$; E_∞ is the equilibrium elasticity modulus; ε_0 is elastic deformation developed at "immediate" loading; J_0 is elastic compliance; ε_∞ is the equilibrium deformation developed at $t \to \infty$; and J_∞ is the equilibrium compliance.

DISCUSSION

IR spectra for all four samples of AA and MBAA copolymer were measured. The measurements were performed not in gels containing just a small amount of the network polymer, but in completely dried samples. This was made for the purpose of approaching 100% concentration of the copolymer in the samples. Because samples of polymers from dried gels represented a very hard glassylike mass, they were mechanically transformed in powderlike samples, which were used for tableting with KBr or preparing a suspension in paraffin oil. Analysis indicates identity of spectra for every sample, measured with KBr and in paraffin oil. However, because the spectra in paraffin oil contain additional bands attributed to the oil, Figure 1 shows IR spectra for all four samples, measured in tablets with KBr. The data shown indicate that spectra contain wide poorly disposed bands, often typical of network polymers. Based on the suggested scheme of synthesis, samples 1 and 2 must differ by the presence of primary and secondary amino groups in the first sample, in which the amount of secondary groups was low, whereas their quantity in the remain-

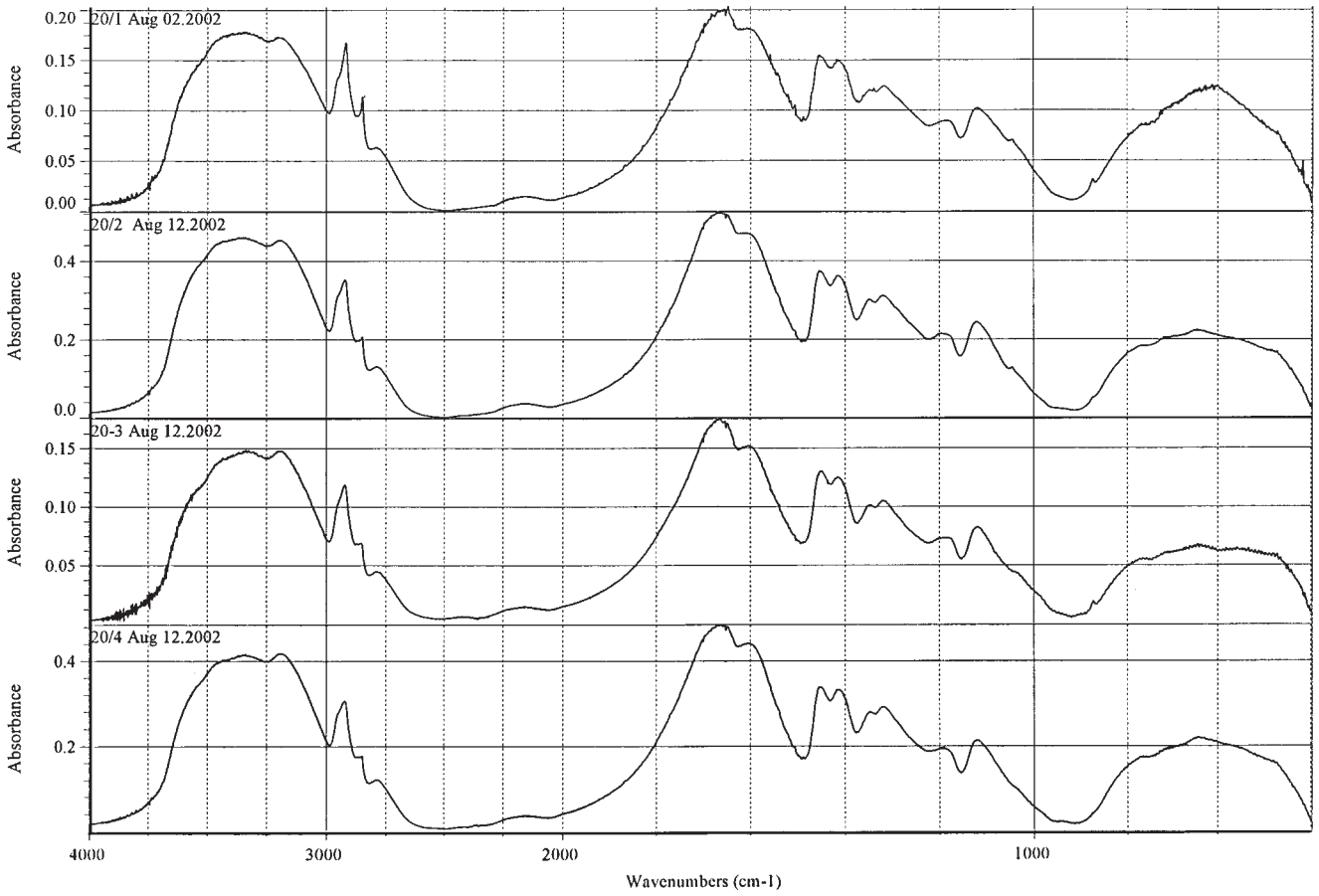


Figure 1 IR spectra for samples 1–4.

ing samples must be noticeable. However, the spectra of all samples are practically identical. A wide and very intense absorption with a complicated profile in the range of 3400–3100 cm^{-1}, attributed to valence oscillations of NH groups, are observed in the spectra. In this connection, it is impossible to clearly determine the shape of spectra in the range of NH groups' valence oscillations, if there are differences in the chemical structure of the studied polymers. The spectra also display an intense amide I band in the area of 1650 cm^{-1}, but the amide II band at 1550 cm^{-1}, which reflects deformational oscillations of NH groups, is absent. This is caused by the network structure of the copolymer, the wide, complex profile of the amide I band, and the possibility of the amide II band being masked behind the wide amide I band.

Samples 3 and 4 differ by conditions and duration of radiation and thermal processing. However, it is known that radiation processing usually changes the physical properties of gels, such as ability to swell, crystallinity variation, and so forth. Such changes may frequently be observed in the spectra; however, these effects are extremely fine and cannot be detected in spectra of polymers possessing very wide degraded bands.

Thermomechanical studies of these four samples were subsequently performed. These studies were used to detect changes of glass-transition temperature of the network polymers, from which hydrogels under consideration are derived. It is known that the glass-transition temperature represents the parameter sensitive to any changes in chemical structure of polymer networks. That is why dry samples, prepared from completely dried gels, were subject to thermomechanical studies.

Figure 2 shows thermomechanical curves for these four samples. The data indicate softening of samples at about 100°C, whereas when approaching 170°C the data display a sharp "negative" deformation, verifying the rapid increase of the sample volume. This type of thermomechanical behavior of polymers was analyzed in detail in the literature.[18,19,76] In the present case, this is induced by high internal stresses occurring during gel drying, which are removed at the glass-transition temperature, and the sample intumesces. After removing internal stresses, the sample deformation increases rapidly, reaching 100% at 280–300°C. Figure 2 also shows that the glass-transition temperature of samples 2 and 4 is somewhat higher than that of the initial sample 1.

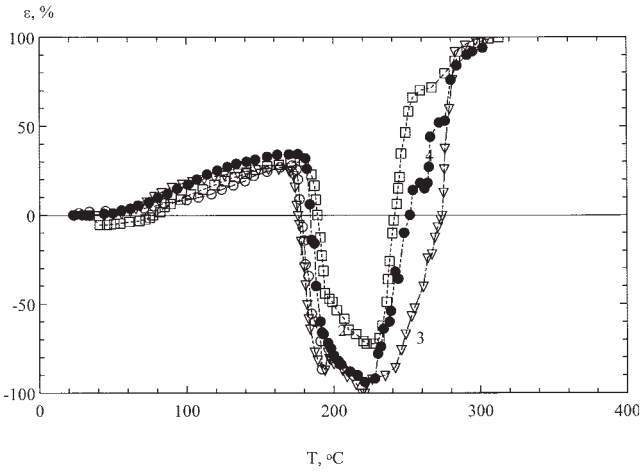


Figure 2 Thermomechanical curves for samples 1, 2, 3, and 4 [(1), (2), (3), and (4), respectively].

For the purpose of analyzing the reasons for changes in chemical structure of the network polymer treated by γ-radiation, the following experiments and calculations were performed. Physical parameters of polyacrylamide were calculated with the help of CHEOPS software (MillionZillion Software Co., Minneapolis, MN). The principle of this software is based on the approach to quantitative analysis of chemical structure effect for linear polymers and polymer networks, discussed in the monographs.[18,19] Usually, the difference between calculated and experimentally determined data does not exceed 3–5%. Table I shows the results of calculations. The calculated parameters were as follows: linear polyacrylamide, homopolymers network derived from MBAA, and two network polymers of different structures. In cases when possible, experimental values of some characteristics are shown in parentheses.

Let us analyze the influence of the chemical structure of current polymeric systems on their properties. The density of polymers is significantly increased with transition from polyacrylamide to a network system derived from MBAA and ahead to networks I and II. Naturally, the glass-transition temperature at the transition from linear polyacrylamide to networks I and II, derived from MBAA, is sharply increased. Conversely, the temperature indicating the beginning of intense thermal degradation does vary weakly, but it thus is much lower than the glass-transition temperature, which, as a consequence, cannot be measured experimentally for these networks because the latter degrade at a much lower temperature. Such experimental assessment can be carried out based only on the analysis of the glass-transition temperature dependency on the composition of copolymers, which can be synthesized and tested. The procedure of this analysis is described in detail in the work by Vinogradova et al.[77]

The surface energy is low, depending on the structure of these polymers, and molar cohesive energy is naturally increased at the transition from polyacrylamide to network systems because the molar volume of the repeating unit becomes much higher. For the specific cohesive energy (the solubility parameter of Hildenbrand), it remains almost unchanged during the transition from polyacrylamide to a network polymer derived from MBAA, decreases at transition to network I, and significantly increases during the transition to network II. This is of great importance for estimation of the polymer interaction with a solvent, which can be water, in particular.

The part of energy of hydrogen bonds, dipole–dipole interaction, and the energy of dispersion interaction in the full cohesive energy are approximately the same for polyacrylamide, the network derived from MBAA, and the network II, although the part of energy of hydrogen bonds and dipole–dipole interaction is much lower for network I and, consequently, the part of dispersion interaction is higher. Thus compared with network I, polyacrylamide and networks derived from MBAA, as well as network II, possess more significant specific intermolecular interaction and, consequently, they possess higher affinity to water, in which the part of energy of hydrogen bonds is extremely high.

Dielectric constants of polyacrylamide and the network derived from MBAA are approximately the same, whereas for network I this parameter is much lower and for network II much higher. The refraction index remains practically equal for these four polymers. The effective dipole moment increases during the transition from polyacrylamide to the network derived from MBAA and network II, but it is lower for network I rather than for network II.

Data in Table I indicate the glass-transition temperature T_g, calculated for polyacrylamide, is 421 K. Judging by data from the literature, experimental T_g values fall within the range of 421–436 K. In accordance with the data of thermomechanical measurements, the temperature of abrupt deformation change (refer to Fig. 2) is about 443 K, which corresponds to the glass-transition temperature.

Among other properties important for practical application of polyacrylamide, the significant portion of energies of dipole–dipole interaction and hydrogen bonds in the total energy of intermolecular interaction (0.579) should be noted. The part of energy of hydrogen bonds in the total cohesive energy is also high (0.447). The solubility parameter of polyacrylamide is 30.8 J/cm^3 and the surface energy is 50.7 mN/m.

The properties of network polymers I and II, at different crosslinking frequencies, were calculated using the same software program. As an example, at equal concentration (equal crosslinking density), 9 wt %, of networks I and II. The glass-transition temperature calcu-

TABLE I
Properties of Polyacrylamide and the Networks Derived from It[a]

Polymer	Values of polymer properties					
	Unit *MM*	V_m (cm^3/mol)	$\Sigma i\ \Delta V_i$ (Å^3)	ρ (g/cm^3)	T_g (K)	T_d (K)
Polyacrylamide	71.1	56.8	64.3	1.25	421 421–441 (exper.)	569 (exper.)
Network derived from *N,N′*-methylene-bisacrylamide	154	119	134	1.30	677	562
Network I	125	93.2	105	1.34	913	589
Network II	140	104	104	1.35	828	551

Polymer	Values of polymer properties						
	γ (dyn/cm)	E^* (kJ/mol)	α_h (rel. un.)	α_{dd} (rel. un.)	α_d (rel. un.)	ε (rel. un.)	E (kJ/mol)
Polyacrylamide	50.7	54.0	0.447	0.185	0.368	4.95	76.9
Network derived from *N,N′*-methylene-bisacrylamide	49.9	111.0	0.433	0.179	0.388	4.93	260
Network I	54.7	73.60	0.328	0.271	0.401	4.40	267
Network II	55.3	107.0	0.449	0.186	0.365	5.70	272

Polymer	Values of polymer properties					
	E_{dd+h} (kJ/mol)	E_d (kJ/mol)	δ $(J/cm^3)^{1/2}$	n (rel. un.)	ΔU (kJ/mol)	$\alpha_G \times 10^{-4}$ (K^{-1})
Polyacrylamide	44.6	32.3	30.8	1.52	91.00	2.28
Network derived from *N,N′*-methylene-bisacrylamide	177	83.3	30.6	1.54	146	1.42
Network I	196	70.6	28.1	1.54	197	1.05
Network II	204	68.80	32.1	1.56	179	1.16

Polymer	Values of polymer properties					
	$\alpha_L \times 10^{-4}$ (K^{-1})	n_e (rel. un.)	$C_p^{\ s}$ ($J/mol^{-1}/deg^{-1}$)	$C_p^{\ l}$ ($J/mol^{-1}/deg^{-1}$)	R ($J/mol^{-1}/deg^{-1}$)	M_e ($J/mol^{-1}/deg^{-1}$)
Polyacrylamide	5.73	299	98.2	191	17.3	21,200
Network derived from *N,N′*-methylene-bisacrylamide	3.56	—	197	329	37.4	—
Network I	2.64	—	152	240	29.1	—
Network II	2.91	—	174	327	33.6	—

[a] *MM* is the molecular mass of the repeating unit of the linear polymer or the repeating fragment of the network; V_m is the molar volume; $\Sigma_i\ \Delta V_i$ is the van der Waals volume of the repeating fragment, ρ is the density T_g is the glass-transition temperature; T_d is the onset temperature of intense thermal degradation; γ is the surface energy; E^* is the cohesive energy; α_h is the relation of hydrogen bonds to total cohesive energy; α_{dd} is the relation of dipole–dipole interaction energy to total cohesive energy; α_d is the relation of dispersion interaction energy to total cohesive energy; ε is the dielectric constant; E is the total energy of intermolecular interaction; E_{dd+h} is the energy of dipole–dipole interaction and hydrogen bonds; E_d is the energy of dispersion interaction; δ is the solubility parameter; n is the refractive index; ΔU is the activaion energy of low-temperature γ-transition; α_G is the coefficient of thermal expansion in the glassy state; α_L is the coefficient of thermal expansion in the rubbery state; n_l is the polymerization degree of polymer when the robbery state appears; c_p^s is the molar heat capacity in the glassy state; c_p^l is the molar heat capacity in the rubbery state; R is the molar refraction; M_l is the molecular mass of polymer when the rubbery state appears; P is the polarizability; C_σ is the stress-optical coefficient; μ is the dipole moment; P_{O_2}, P_{CO_2}, and P_{N_2} are the permeabilities by oxygen, carbon dioxide, and nitrogen, respectively (the unit of measurement of permeability 1 DU = 0.45×10^{-10} cm^2 s^{-1} atm^{-1}).

lated for the current concentration of suggested networks increased by 11° for network I and by 10° for network II (note that the glass-transition temperature calculated for the network, derived from MBAA at its 1 wt % concentration, is 422 K). Figure 3(a) and (b) show dependencies of the glass-transition temperature on the

TABLE II
Stress Relaxation Parameters Evaluated by Approximation

Memory function $T_1(\tau)$						
Sample no.	E_0 (Pa)	E_∞ (Pa)	r	k^* (min^{-1})	β	$A_1 \times 10^{-23}$ (deg m^{-3} J^{-1})
1	17,320	9,254	0.98	0.1	0.2	1.564
2	19,680	10,277	1.00	0.1	0.6	1.515
3	14,020	9,425	0.98	0.1	0.3	2.208
4	12,770	11,520	0.98	0.01	0.4	7.451
Memory function $T_2(\tau)$						
Sample no.	E_0 (Pa)	E_∞ (Pa)	r	a	γ	$A_2 \times 10^{-23}$ (deg m^{-3} J^{-1})
1	11,380	11,060	0.89	0.0209	0.5	25.58
2	11,260	11,080	0.72	0.0209	0.5	50.02
3	10,190	10,060	0.85	0.0209	0.5	57.86
4	11,980	11,925	0.94	0.0494	0.4	159.0

composition of network polymers, synthesized by schemes I and II. Clearly if the crosslinking density would be significantly increased by γ-radiation treatment, the glass-transition temperature (T_g) of such network systems should increase sharply. However, judging by the experimental data, the increase of T_g is rather low, observed for samples 2 and 4 only. Thus if under the effect of γ-radiation crosslinking does happen, it is very low.

Then dependencies of the glass-transition temperature of cyclic polymers containing different quantities of suggested structure III were calculated. The calculation results are shown in Figure 3(c) in the form of T_g dependencies on the content of structure III. It is clear that if the quantity of structure III formed would be noticeable, the glass-transition temperature of such a system should be sharply increased. However, judging by the experimental data (Fig. 2), processing of the initial copolymer at 120°C and under 1.2 atm pressure for 40 min (sterilization) scarcely changes this parameter.

For a more detailed analysis of the network structures subject to γ-radiation treatment, their ^{1}H- and ^{13}C-NMR spectra were analyzed. The proton NMR (PMR) spectrum of the initial polyacrylamide in D_2O (Fig. 4) possesses two groups of broadened signals at 1.5 and 2.0 ppm, with 2 : 1 integral intensities attributed to CH_2 –and CH fragments of the polymer. Apparently, broadening of the signals is associated with steric stress in the polymer, which induces somewhat different magnetic environments of these fragments resulting from anisotropic effects of neighboring groups. The PMR spectrum also reveals broadened signals of much lower intensity at 6.7 and 7.4 ppm, which may be attributable to olefin protons of the initial monomer and to polymerization products. Concentration of these protons does not exceed 3%.

TABLE III
Creep Parameters Evaluated by Approximation

Memory function $T_1(\tau)$						
Sample no.	$J_0 \times 10^4$ (Pa^{-1})	$J_\infty \times 10^4$ (Pa^{-1})	r	k^* (min^{-1})	B	$A_1 \times 10^{-23}$ (deg m^{-3} J^{-1})
1	5.587	14.600	1.0	0.00001	0.2	0.4486
2	8.776	10.331	0.99	0.001	0.2	4.051
3	6.904	10.984	0.99	0.01	0.3	1.229
4	5.857	9.063	0.99	0.00001	0.8	1.317
Memory function $T_2(\tau)$						
Sample no.	$J_0 \times 10^4$ (Pa^{-1})	$J_\infty \times 10^4$ (Pa^{-1})	r	a	Γ	$A_2 \times 10^{-23}$ (deg m^{-3} J^{-1})
1	7.998	9.919	0.98	0.169	0.2	3.012
2	9.474	10.141	0.99	0.139	0.2	10.016
3	9.302	9.540	0.95	0.0209	0.5	29.57
4	6.730	6.841	0.96	0.0209	0.5	43.87

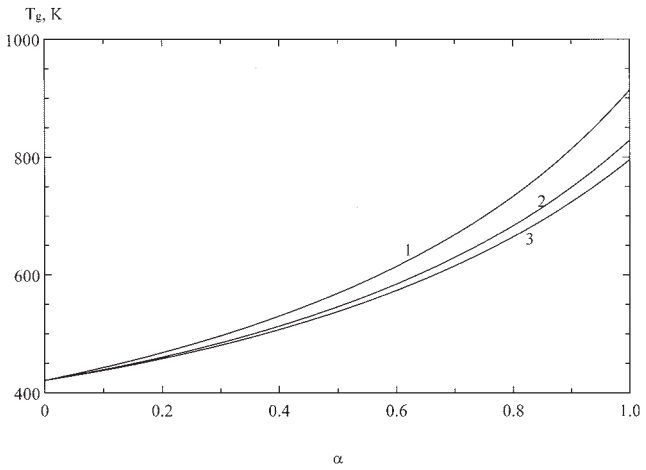


Figure 3 Dependencies of glass-transition temperature T_g on molar parts of networks I (1) and II (2), and cyclic structure III (3).

For signals assignment, the ^{13}C-NMR spectrum of initial polyacrylamide in D_2O (Fig. 5) was measured under *J*-modulated nuclear spin-echo mode. It contains the series of ^{13}C signals of CH_2 groups at 36–38 ppm, the series of ^{13}C signals of CH groups at 43.5–44.3 ppm, and a broadened signal at 182 ppm attributed to nuclei of carbonyl carbons in the polymer.

Because short-term γ-radiation scarcely induces changes in the spectra, a more detailed study was performed, which gave an opportunity to detect changes in the structure of the polymers under investigation. After a 2-h exposure to radiation, NMR spectra were observed to change significantly, which may prove the occurrence of structural changes during radiation.

Figure 6 shows the ^{13}C-NMR spectrum of the long-term radiated sample, recorded in the usual mode, clearly showing the occurrence of broad shoulders in the basement of a group of signals from carbon nuclei of the CH group at 44 ppm. The presence of these shoulders indicates the occurrence of new CH groups in a different structural environment compared with the initial polymer. The spectrum also reveals an extremely broad signal centered at 120 ppm, the occurrence of which can be stipulated by partial content of alkene structures, formed during long-term exposure to γ-radiation. The presence of such structures can be caused either by formation of free acrylic acid (attributed either to long-term exposure to γ-radiation) or by hydrogen release from repeating units of polyacrylamide backbone and their transformation in accordance with the following scheme:

For the purpose of analyzing the mechanism of alkene structure formation the gel containing 3.5% of MBAA was primarily added by 5% of acrylic acid, after which the ^{13}C-NMR spectrum of this system was recorded. It

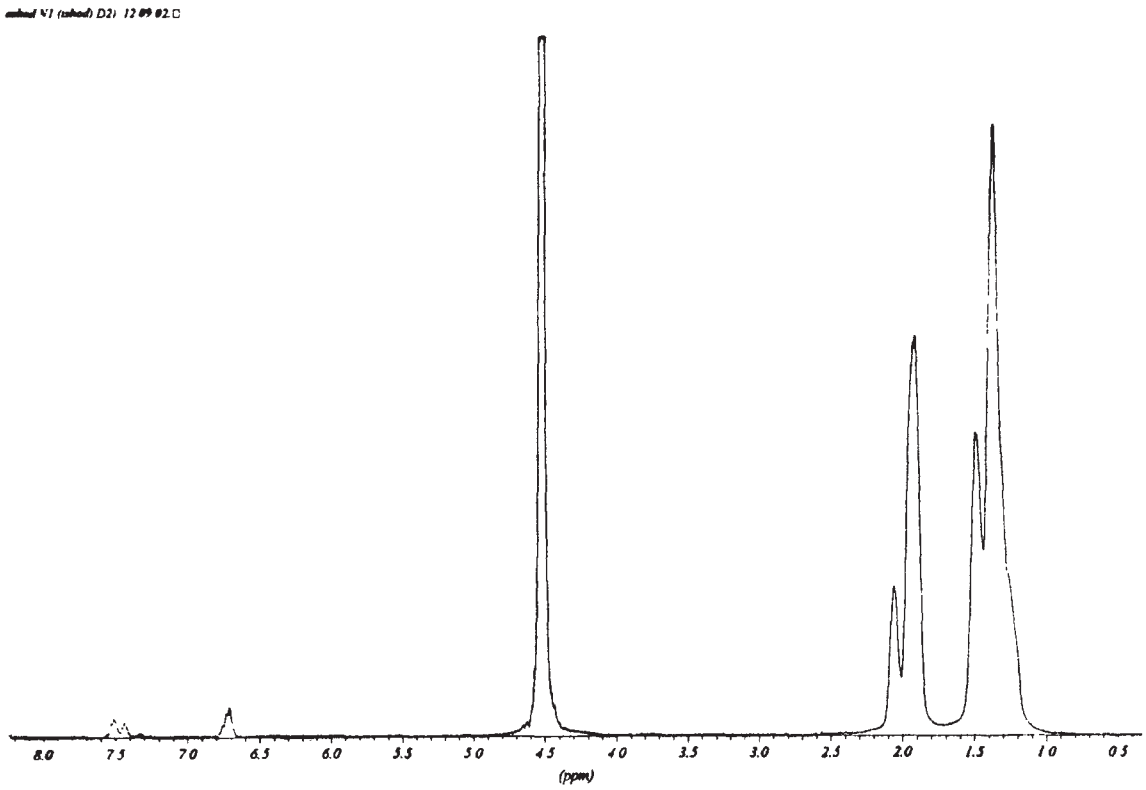


Figure 4 PMR spectrum of primary gel derived from polyacrylamide in D_2O.

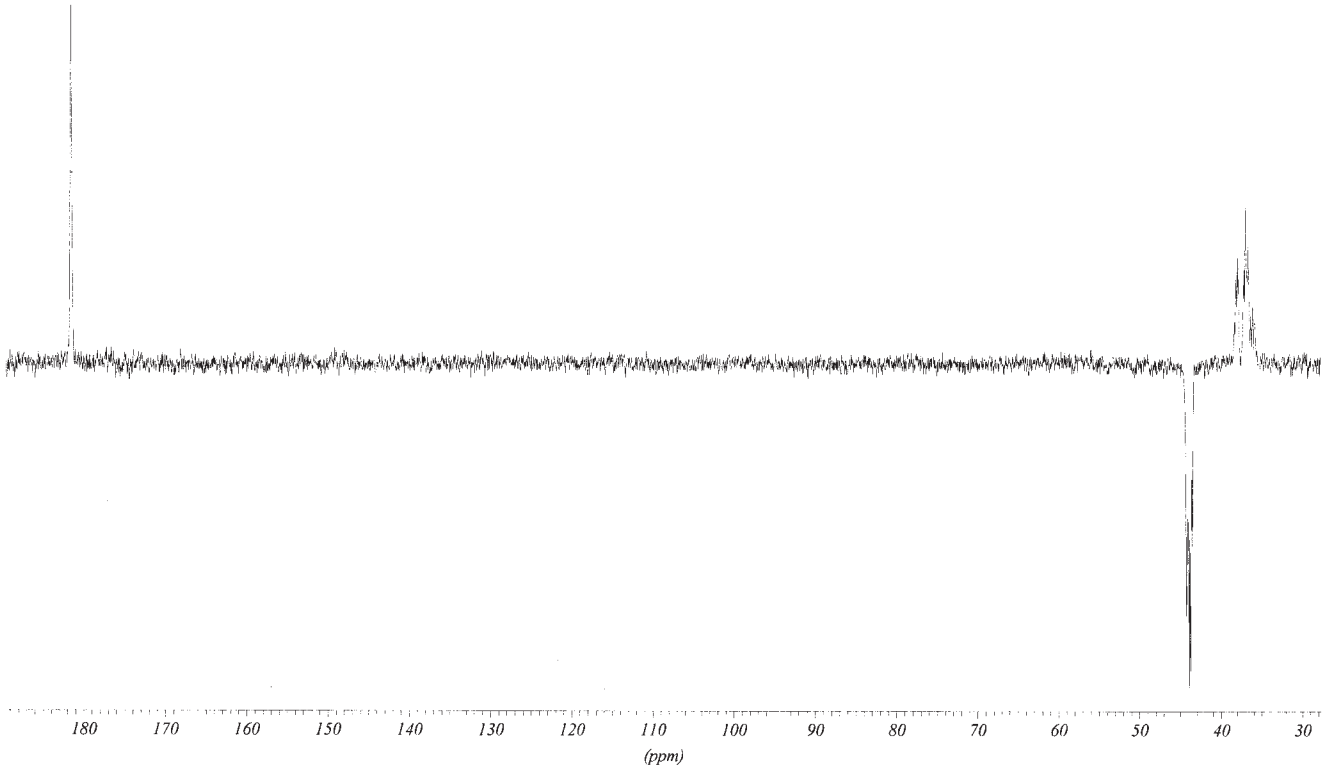


Figure 5 ^{13}C-NMR spectrum of primary gel derived from polyacrylamide in D_2O, measured under conditions of *J*-modulated nuclear spin echo.

was found that the spectrum of such a gel possesses no shoulders, similar to the ones observed, and the entire spectral image remains unchanged. As a consequence, the occurrence of a broad signal can be attributed to formation of structures with respect to the above-shown scheme.

Thus both experiments and calculations indicate polymerization of residual monomers present in the initial gel, induced by γ-radiation, as well as formation of a crosslinked network of very low concentration and structures containing >C═C< bonds. It should seem that these structural changes in the polymer are minor. However, they induce noticeable changes in both mechanical properties of these gels and their behavior in proteic tissues. This is indicated by results of relaxation measurements and both morphological and histological studies, shown below.

The results of stress relaxation and creep measurements will be discussed first. Figure 7 presents stress relaxation and creep curves. Clearly, Figure 7(a) shows that the initial sample 1 displays the highest ability to stress relaxation because relaxing stress in this sample is reduced to a greater extent than that in all other samples. Thus, a tendency to benched relaxation is clearly observed. When sample 1 was washed and exposed to further γ-radiation the relaxation depth was noticeably reduced [Fig. 7(a), curve 2], which is associated with additional crosslinking. After autoclaving at an increased temperature the gel becomes more flexible [Fig. 7(a) and (b), curve 3], stress relaxation proceeds more profoundly and creep is developed more intensively than in all other samples. Finally, the gel subject to additional γ-radiation displays a lower ability to stress relaxation and creep [Fig. 7(a) and (b), curve 4, sample 4].

Let us analyze changes in physical parameters of gels, determined by approximation of stress relaxation and creep curves. Table II shows results of approximation of stress relaxation curves for the four samples, performed using the software program presented in the literature.[18,19] Table II indicates a correlation index approaching 1 in the case of the memory function $T_1(\tau)$ application, whereas the memory function $T_2(\tau)$ displays an index much lower than 1. From the above-discussed positions this means that for the studied hydrogels the limiting stage of relaxation is the rate of relaxants' interaction, and diffusion of the products formed (nonrelaxants) proceeds at a greater rate and does not limit the general process of relaxation: this is quite natural because the viscosity of hydrogels is low compared with that of solid polymers, and diffusion in the hydrogels proceeds at a high rate. The primary elasticity modulus E_0 is increased by radiation treatment of sample 1, which is caused by postpolymerization and an additional crosslinking process (sample 2). Therefore, it is decreased during autoclaving (sample 3). For the same

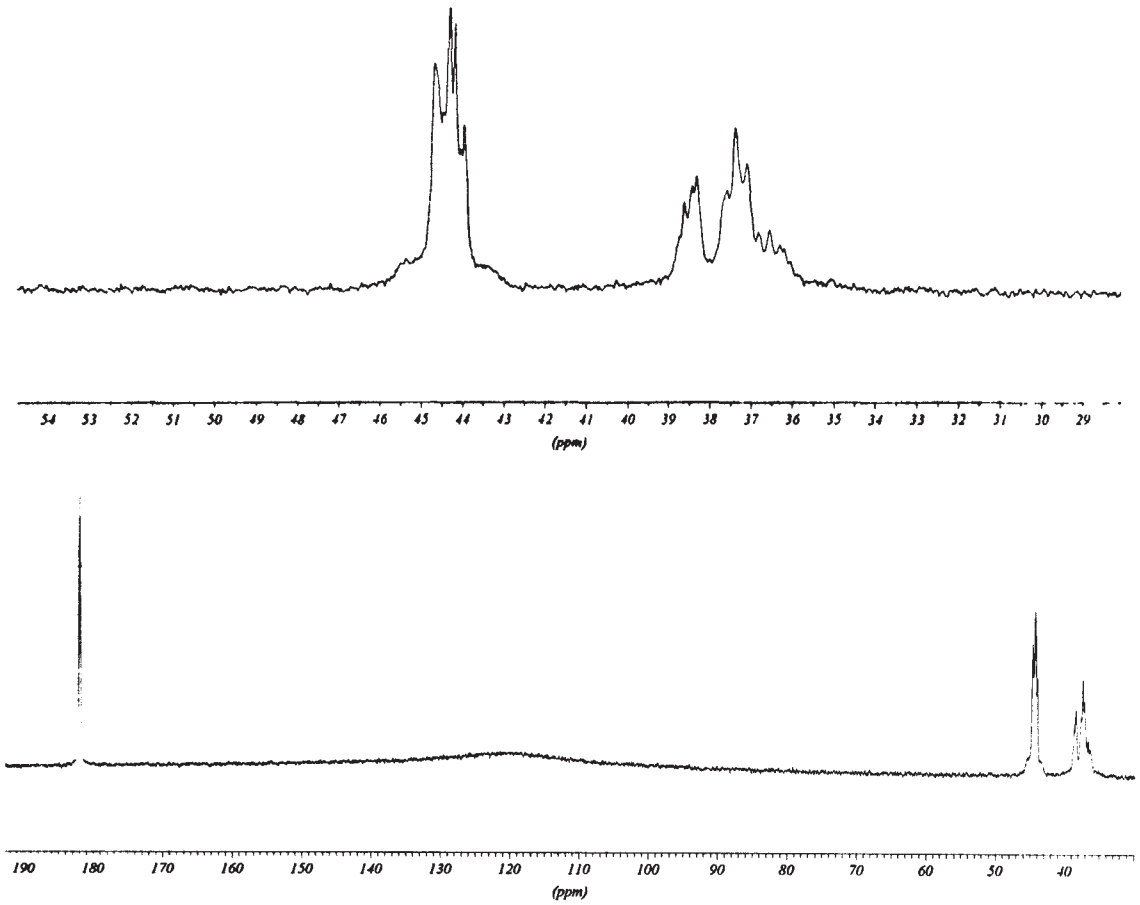
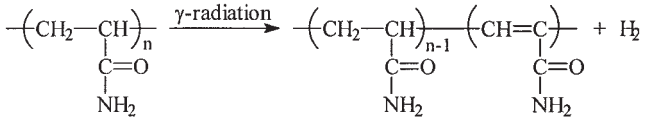


Figure 6 ^{13}C-NMR spectrum of the gel after long-term exposure to γ-radiation.

reasons, the quasi-equilibrium modulus E_∞ is also increased during the transition from sample 1 to sample 2, but is decreased by autoclaving. For the purpose of improving mechanical properties of this sample, it was treated by additional γ-radiation that increases quasi-equilibrium modulus E_∞ to its maximum. The value k^* proportional to the rate constant of relaxants' interaction is the same for samples 1–3, but decreases for sample 4, which is also obligate and associated with the gel density increase.

Data obtained by approximation of the creep curves (Table III) are adequate to explain the data on stress relaxation. In the case of the memory function $T_1(\tau)$ application, the correlation index approaches 1, and for $T_2(\tau)$ it is also high but lower than that for the former. That is why the limiting stage of the creep process is also



Scheme 4

the rate of relaxants' interaction. Quasi-equilibrium compliance J_∞ decreases with transition from sample 1 to sample 2, but increases again after autoclaving (sample 3). Then after additional γ-radiation treatment it reaches the minimum (sample 4). Thus after completion of all sterilization procedures and additional γ-radiation treatment the gel obtains the highest elastic properties (its stress relaxation is lower and creep is minimal). This provides a foundation for successful use of hydrogels derived from polyacrylamide for medical purposes, which is proved by the data obtained in the literature.[60,62,78]

Three types of gels, corresponding to samples 1, 3, and 4, were used in these studies. The tissue reaction to gel implantation was studied in experimental–morphological and clinical–morphological investigations. In the experiment, the gel was implanted hypodermically and intramuscularly by injections. In clinics, morphological studies were performed with three observations: a month after facial hypodermic implantation and 6 and 6.5 months after implantation—for the purpose of increasing mammaplastics by filling in a fibrotic capsule after prosthesis removal.

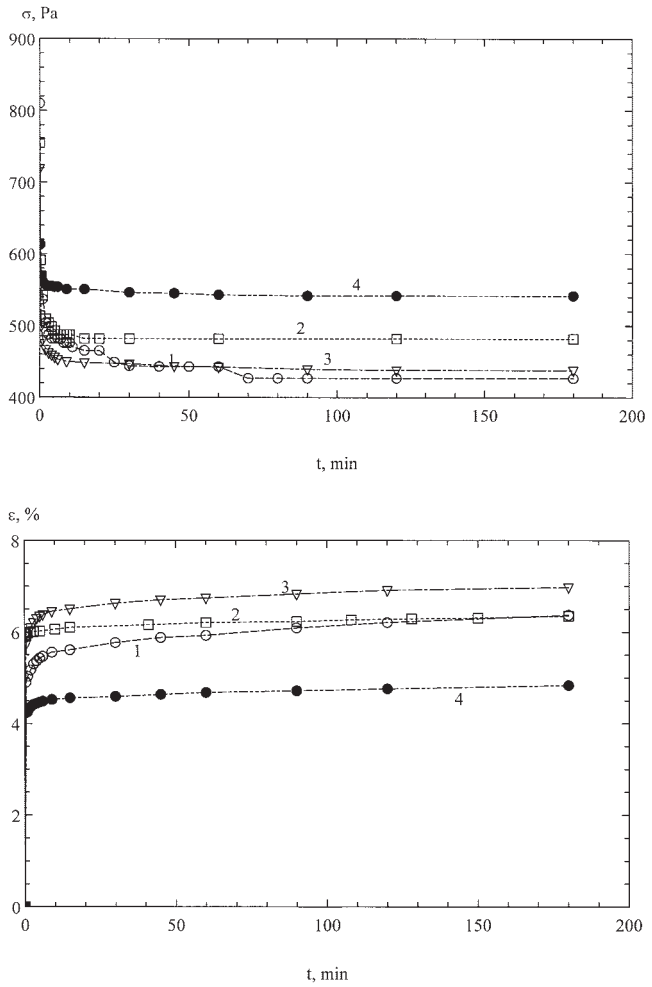


Figure 7 Stress (a) and creep (b) relaxation curves, measured during axial compression. Curve numbers correspond to sample numbers.

For the gel of form 1, investigation results indicate that 7 days after implantation the capsule around the gel consisted of immature granulation tissue, in which inflammatory reaction in the form of an edema, neutrophilic lymphocytic infiltration expressed by the macrophage reaction, was detected. Proliferation of fibroblasts and maturation of connective tissues were retarded. Up to 14th day the capsule was of the same thickness, and maturation of the granulation tissue was insignificant; neutrophilic inflammatory reaction and numerous macrophages resorbing the gel remained. After 36 days the whole implant was characterized by cellular conglomerates alternating with aggregates of large foamy macrophages. The capsule of connective tissue was thick, consisting of sclerosal tissue, in which chronic inflammatory infiltration was preserved.

Thus the gel of form 1 used in these tests promoted expressed and prolonged aseptic inflammatory reaction and comparatively rapid macrophage resorption.

Morphological study of the tissue reaction of the organism to inflammation of hydrogel samples 3 and 4 shows[60,78] that this reaction is minimal, although these samples display somewhat different tissue reactions. For sample 4, in early stages (3–7 days after implantation) it is limited by weak lympho-macrophage infiltration with single neutrophils and weak edema of the tissue, which confirms minimal inflammatory reaction. Thirty days after implantation capsule A remains thin. It consists of mature connective tissue, the amount of fibroblasts in which is decreased, and RNA concentration in the rest cells is also decreased. In encapsulated zone B hydrogel fragments C remain, resorbed by macrophages. The internal surface of the capsule is partly lined by macrophages. In later stages (60 and 90 days) the capsule structure does not change; at places small fibrotic folds of fibroblasts penetrated from the capsule inside the gel.

Clinical–morphological observations, performed after injection of 90 mL of hydrogel 4 for the purpose of dermatension of a skin–fatty flap on the face with future cicatrix plastics, show that an extremely thin and loose connective capsule, consisting of just several layers of collagen fibers and fibroblasts, was formed at the interface between hydrogel and tissues. Cellular lympho-macrophage infiltration is minimal. In some areas, outside the capsule tissue, vacuoles that remained at places of resorbed gel were observed. A weakly expressed macrophage and giant-cell reaction was observed there.

Thus the tissue reaction principally depends on the method of gel production, all other factors being equal (e.g., water content in gel, crosslinking agent concentration in synthesis). In the present tests the gel of form 1 initiated much higher expressed inflammatory reaction, more intensive macrophage resorption, and high-velocity growing through by the self-organism tissue. This is caused by the faster and massive breakage of intermolecular bonds in the gel and disruption of a structure by macrophages and neutrophils deeply penetrating into the gel in this case. Conversely, gels of forms 20/3 and 20/4, subject to autoclaving and additional γ-radiation, gave the minimal tissue reaction to implantation, especially the gel of form 20/4. Inflammatory reaction in early stages was extremely low, fibroblast reaction was weakly expressed, and at late stages the capsule became thin. Gel resorption by macrophages and its growing through connective tissue were extremely slow and proceeded in the near-capsule layer only.

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