Preparation and Characterization of Polyacrylamide Cryogels Produced from a High-Molecular-Weight Precursor. II. The Influence of the Molecular Weight of the Polymeric Precursor

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ABSTRACT: Polyacrylamide gels and cryogels were prepared by the crosslinking reaction of polyacrylamide (a polymeric precursor) with glutaric aldehyde (a crosslinking agent) in liquid and moderately frozen aqueous media, respectively. Polymeric precursors of different viscosity-average molecular weights (0.3, 1, 3, and 9 MDa) were used. The molecular weight of the precursors, as well as the reaction temperature and concentration of the crosslinking agent, exerted a pronounced influence on the efficiency of gelation (gel fraction yield) and on the properties (swelling capability) and structural peculiarities of the resulting gels (reference samples) and cryogels. The highest efficacy was inherent in the cryotropic gelation process when the polymeric precursor had a molecular weight of about 3 MDa, whereas the implementation of polyacrylamides of lower (0.3 or 1 MDa) or higher (9 MDa) molecular weights diminished the gel formation efficiency. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 382–390, 2008

Key words: polyacrylamide; cryogel; crosslinking

INTRODUCTION

Polymeric cryogels are macroporous gel matrices that are prepared in moderately frozen media.[1] Similar to traditional gels, that is, those formed in liquid media (without any freezing), cryogels can have covalent interchain crosslinks in the junction knots of a three-dimensional (3D) network, can include stable noncovalent (physical) interchain links (e.g., hydrogen bonds and cooperative hydrophobic interactions) in the knots, or can contain ionic/coordination bonds between the macromolecules in respective networks and also can include mixtures of such crosslinks in the structure of the gel phase. The physicochemical and mechanical properties of polymeric cryogels, their macroporosity characteristics, and their resulting fields of application are determined by the properties of the gel precursors, their concentration in a feed to be frozen and thawed, and, naturally, the conditions of the cryogenic process. Diverse aspects of cryotropic gelation phenomena and the applied potential of cryogels have been already discussed in a series of review articles.[1–7]

Among covalent cryogels, polyacrylamide (PAAm)-based ones prepared through the cryogenic copolymerization of acrylamide (AAm) and *N*,*N*′-methylene-bisacrylamide in not deeply frozen aqueous media have been rather well studied,[1] whereas polyacrylamide cryogels (cryo-PAAGs) synthesized from a high-molecular-weight precursor, that is, linear PAAm, by chemical crosslinking was for the first time described only in our recent work.[8] In that study, we determined the conditions for the preparation of such novel cryo-PAAGs via the crosslinking of PAAm with glutaric aldehyde (GA) in alkaline aqueous solutions of the polymer and revealed the influence of the initial PAAm/GA ratio and freezing temperature on the gel fraction yield, swelling parameters, and morphological peculiarities of respective cryogels produced from such a polymeric precursor. However, this was done with a PAAm sample of only one molecular weight, so the effects that molecular weight characteristics of PAAm could exert on the properties of resulting cryo-PAAGs were still unclear. Therefore, the aims of this study were to reveal such effects and to investigate in detail the influence of the precursor's molecular

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weight on the efficiency of cryotropic gel formation and the properties of the resulting cryogels.

EXPERIMENTAL

Materials

AAm, ammonium persulfate (APS), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) were purchased from Aldrich (St. Louis, MO), and a 50% aqueous solution of glutaraldehyde was acquired from Fluka (Buchs, Switzerland).

Synthesis of the linear PAAm

The polymeric precursors, further used for the preparation of polyacrylamide gels (PAAGs) and cryo-PAAGs, were synthesized according to an earlier described procedure.[9] In brief, a 1*M* monomer aqueous solution was deaerated by argon bubbling (10 min) and then chilled in an ice bath to 1–2°C. The required amounts of TMEDA and APS were added; afterward, the reaction mixture was frozen in the chamber of an NCB-3100 cryostat (Eyela, Tokyo, Japan) at −12, −14, or −18°C to synthesize PAAm samples with viscosity-average molecular weights (M_η) of approximately 9, 3, and 1 MDa, respectively. To prepare the 0.3-MDa polymer, a 0.5*M* AAm solution with TMEDA and APS added was incubated at 35°C with a C-WBE water bath (Chang Shin Science Co., Seoul, Korea). The reaction time in all the cases was 24 h. The frozen samples were thawed at room temperature, whereas the system that was reacted at 35°C was cooled to room temperature. The polymers that formed were separated from the low-molecular-weight admixtures by dialysis through a benzoylated cellulose membrane (Sigma, St. Louis, MO; cutoff limit ≈ 2 kDa) against pure water. PAAm thus conditioned was precipitated with cold acetone and then dried at 50°C and 0.07 MPa with a VO-64 vacuum drying oven (HYSC, Seoul, Korea).

The intrinsic viscosity ([η]) values of the PAAm specimens were measured in a 1*M* $NaNO_3$ solution with an Ubbelohde capillary viscometer at 30°C. M_η of the polymers was calculated with the following equation:[10]

$$[\eta] = 3.73 \times 10^{-4}\, M_\eta^{0.66}\ (\mathrm{dL/g})$$

Chromatographic analysis

A size exclusion chromatography (SEC) analysis of the PAAm samples was carried out at room temperature with a column (10 × 400 mm) packed with CL-Sepharose 2B resin (Amersham Pharmacia Biotech, Uppsala, Sweden), as described elsewhere.[9] The recording of the elution curves was performed with a flow-through refraction unit (Knauer, Berlin, Germany). The eluent liquid was 0.2*M* NaCl, and the elution rate was 60 mL/h.

Preparation of the PAAGs and cryo-PAAGs

Synthesized PAAm samples of different molecular weights were used to prepare PAAGs and cryo-PAAGs. A fixed amount of TMEDA (aprotic base) was added to a 2% solution of the polymer to adjust the pH value up to 10. Upon the synthesis of a cryo-PAAG, the PAAm solution was chilled to 1–2°C, a further necessary volume of a GA solution was added, and the mixture was stirred for 30 s. Then, the system was placed in a cryostat chamber. Upon the preparation of a PAAG, the required amount of GA was added to the PAAm solution, and then the sample was placed in the chamber of a CW-05G thermostat (Jeio Tech, Daejon, Korea). The reaction time in all cases was 24 h. Because the reaction system did not crystallize at −5°C on account of supercooling effects, the specimens, before incubation at this temperature, were quickly (for 15 s) frozen in liquid nitrogen to crystallize the greatest amount of the solvent. Thus, the frozen samples were then transferred to the cryostat with a coolant temperature equal to −5°C. After incubation, the frozen samples were thawed at room temperature and rinsed from soluble admixtures with a large amount of distilled water.

Measurements of the degree of swelling for the gels and cryogels

Measuring the swelling degree of a gel phase was carried out with a known procedure.[11] A swollen specimen was placed on a porous glass filter, closed, and squeezed *in vacuo* for 5 min out of the unbound water. In this time, the unbound water moved away from the spongy specimens. Furthermore, the wet preparation was weighed and then dried in a vacuum drying oven to a constant weight. Because the swollen PAAG did not contain unbound water on account of its microporous structure, it was not necessary to squeeze out such samples with the aforementioned manipulations. The amount of gel-bound water [swelling degree of the polymer phase ($S_{w/w}$)] was calculated with the following formula:

$$S_{w/w}(\mathrm{g\ of\ H_2O/gof\ dry\ gel} = (m_{ws} - m_{ds})/m_{ds},$$

where m_{ws} is the mass of the wet sample and m_{ds} is the mass of the dried sample, respectively.

Microscopic investigations

The preparation of the cryo-PAAG samples for scanning electron microscopy (SEM) studies was accomplished essentially in accordance with a procedure

published elsewhere.[12] Chemical fixing of the cryogel specimens was carried out by their immersion in a 2.5% solution of GA in a 0.2*M* Na phosphate buffer (pH 7.5) at 25°C for 3 h. Furthermore, the specimens were rinsed with water and placed in aqueous solutions of ethanol with concentrations of 30, 50, 70, 80, 90, and 95% for 15 min each and then in isoamylacetate for 20 min (two times). Then, these samples were transferred to an HCP-2 critical point dryer (Hitachi, Tokyo, Japan) and dried. The dried specimens were coated with silver paste, and the morphology of the cryogels was studied with an S-4100 scanning electron microscope (Hitachi).

RESULTS AND DISCUSSION

Preparation and characterization of the polymeric precursors

The synthesis of various covalent gels through the chemical (or radiation) crosslinking of polymer precursors in a solution is one of two main approaches to preparing such gel matrices; the second one is the formation of 3D networks through the polymerization or polycondensation of corresponding monomeric precursors.[13,14] Because the properties and structure of gel systems are acquired during their formation, the precursor's characteristics (chemical nature, chain length, conformation of macromolecules, reaction ability, etc.) and the conditions of gel formation (solvent, concentration of reagents, temperature, and time) are the governing factors. Therefore, in the case of gels prepared by the crosslinking of polymeric precursors, the molecular weight of the initial soluble polymer very often exerts an essential influence on the properties of solvated networks thus prepared.[15–18] When we are dealing with the cryotropic gelation processes occurring in moderately frozen media, the phase state of the reaction system (whether it is in the pre-eutectic or posteutectic regions of a state diagram) and, in some cases, the regimes of the freezing and thawing of specimens are additionally included with the aforementioned factors.[1]

Inasmuch as the main problem considered in this work was the elucidation of the influence of the molecular weight characteristics of PAAm on the properties and structure of the resulting cryogels, at first we synthesized and characterized a series of linear PAAm samples. Afterwards, they were used as polymeric precursors for the preparation of PAAGs and cryo-PAAGs.

TABLE I
Polymerization Conditions and M_η Values of Synthesized PAAm Samples Further Used as Polymeric Precursors in the Preparation of PAAGs and Cryo-PAAGs

Concentration of AAm in the feed (mol/L)	Reaction temperature (°C)	M_η (MDa)
0.5	+25	0.3 ± 0.01
1	−12	8.7 ± 0.40
1	−14	2.9 ± 0.15
1	−18	1.0 ± 0.02

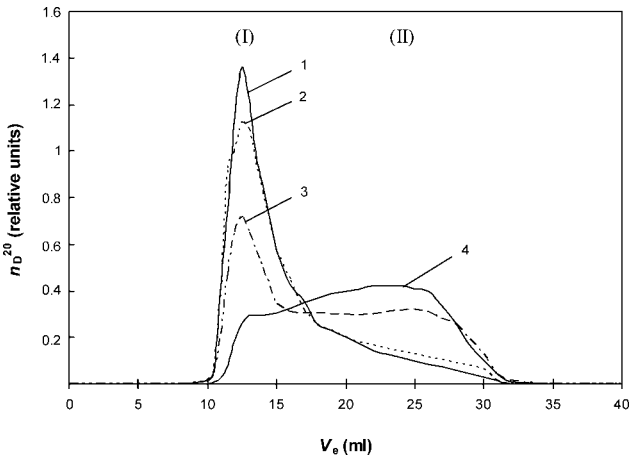

Figure 1 SEC curves of PAAm samples with approximate M_η values of (1) 9, (2) 3, (3) 1, and (4) 0.3 MDa.

These PAAm specimens of different molecular weights were synthesized by the redox-initiated cryopolymerization of AAm with methods described in detail elsewhere.[9] The polymers after their isolation and purification (see the Experimental section) were analyzed with the aid of capillary viscometry and SEC. The data on the conditions of the PAAm synthesis and on the characteristics of the polymers thus obtained are collected in Table I.

Because the cryopolymerization of AAm in a not deeply frozen aqueous medium allows not only the preparation of PAAm specimens of a very high molecular weight ($M_\eta > 10^6$ Da) but also the variation of the reaction temperature[9,19–21] and the control of the molecular weight characteristics of the final polymer, we used this approach for the synthesis of polymeric precursors of high M_η values: ~ 9, ~ 3 and ~ 1 MDa. In addition, PAAm with $M_\eta \sim 0.3$ MDa was synthesized by the polymerization of AAm in solution at 35°C. Figure 1 presents the SEC profiles of all these polymers. V_e in X axis and n_D^{20} in Y axis are the elusion volume and index of reaction, respectively. Such chromatographic profiles are well known[22] to reflect the molecular weight distribution (MWD) of the respective polymers.

The PAAm sample with $M_\eta \sim 9$ MDa (curve 1) had the narrowest MWD and contained the largest amount of the highest molecular weight fraction (I). In the samples of a lower molecular weight (curves 2–4), the content of fraction I was notably decreased, and the amount of fraction II was increased. Besides, one also can see changes in the MWD from a nearly

unimodal distribution (curve 1) to a bimodal distribution (curves 2–4). The data on the approximate molecular weights of fractions I (~ 2 MDa) and II (~ 0.9–0.3 MDa) were taken from the results of the calibration of a column.[21]

The PAAm specimens thus prepared and characterized were then used in the syntheses of PAAGs at a positive temperature and in the syntheses of cryo-PAAGs at various negative temperatures.

Synthesis of PAAGs and cryo-PAAGs from PAAm precursors of different molecular weights

One of the well-recognized features of the processes of cryotropic gel formation is the effect of an apparent decrease in the critical concentration of gelation (CCG) in comparison with the gel formation of the same initial feed in liquid media at temperatures above the crystallization point of such a system.[1] The main reason for this effect is the phenomenon of an increase in the solute concentration (the cryoconcentrating effects) when, upon the freezing of the initial precursor solution, the major portion of the solvent is crystallizing. Because in such a macroscopically frost-bound reaction bulk the cryogel is formed in the small unfrozen areas [the so-called unfrozen liquid microphase (ULMP)],[23] an increased concentration of reagents significantly promotes the gelation despite the low temperature and very high viscosity of ULMP. As a result, opportunities for obtaining cryogels at lower initial concentrations of precursors arise, and this effect is manifested as an apparent decrease in CCG or, in other words, as an enhancement of the efficiency of gelation. Rather similar effects were also observed in this work for cryo-PAAG formation upon PAAm crosslinking with a decreasing amount of GA.

Thus, Figure 2 shows the dependence of the gel fraction yield on the $CONH_2$/CHO molar ratio for amide and aldehyde groups of PAAm and GA, respectively, in the initial reaction system for the PAAG samples synthesized at 25°C with a fixed concentration (2%) of polymers of different molecular weights, and Figure 3 presents the same dependence for cryo-PAAGs synthesized at −5, −10, −15, and −20°C. In these experiments, the ratio of the gel-forming polymer (PAAm) to the crosslinking agent (GA) was varied from 2.5 to 60 mol of amide groups/mol of aldehyde groups.

Although upon gelation in solution at 25°C PAAGs were formed at the lowest GA concentrations corresponding to $CONH_2$/CHO molar ratios equal to 40 : 1 (PAAm with $M_\eta \sim 3$ MDa), 30 : 1 (PAAm with $M_\eta \sim 9$ MDa), 10 : 1 (PAAm with $M_\eta \sim 1$ MDa), and only 2.5 : 1 (PAAm with $M_\eta \sim 0.3$ MDa; Fig. 2), PAAm crosslinking with GA in moderately frozen media allowed the formation of cryogels at a considerably lower GA initial concentration for all the polymeric precursors used in this study (Fig. 3). Without a doubt, this result was the manifestation of the aforementioned effect of an apparent decrease in CCG inherent in cryotropic gelation in general.[1]

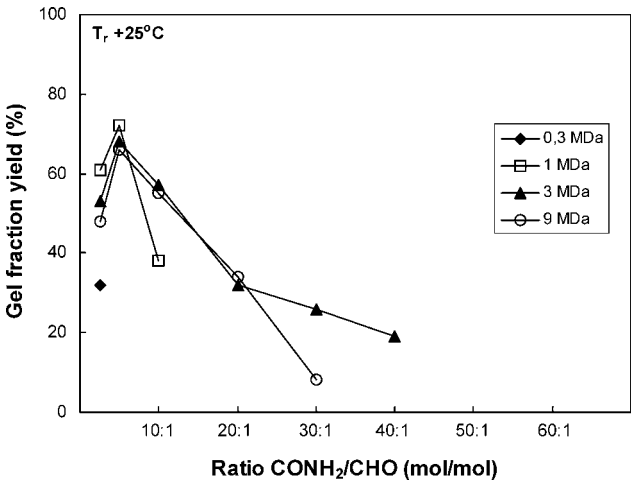

Figure 2 Dependence of the gel fraction yield on the $CONH_2$/CHO molar ratio in the initial reaction solution for PAAG samples synthesized at +25°C.

In the case of cryo-PAAG formation, the values of these lowest boundary GA concentrations depended on both the reaction temperature and the molecular weight of the polymeric precursor. Over the temperature range from −5 to −20°C that we used in the experiments on the preparation of cryogels, the latter were formed from all the PAAm representatives and at all the $CONH_2$/CHO ratios only at −5°C [Fig. 3(a)]. Upon gel formation at −10°C, employing PAAm with $M_\eta \sim 0.3$ MDa, we were able to obtain cryo-PAAGs, already with a markedly lower yield, and the boundary $CONH_2$/CHO molar ratio was 40 : 1 [Fig. 3(b)] versus 60 : 1 in the previous case [Fig. 3(a)]. Further lowering the reaction temperature to −15 or −20°C [Fig. 3(c,d)] did not allow the preparation of cryo-PAAGs at all when such a polymeric precursor of the lowest molecular weight (among those implemented in this study) was used, and there was a shift to a higher GA concentration (i.e., the boundary $CONH_2$/CHO ratio shifted to 40 : 1) for PAAm with $M_\eta \sim 1$ MDa.

These data support the idea that among the examined conditions, the most favorable ones for the formation of a 3D network of cryo-PAAGs existed at −5°C, and upon PAAm crosslinking with GA in such moderately frozen systems, the molecular weight of the polymeric precursor was of significance for the results of cryotropic gel formation. The latter fact was also pointed out earlier in a study on the preparation of cryogels of crosslinked chitosan when three polymers with M_η values of 0.097, 0.349, and 0.881 MDa were cured with GA in a frozen

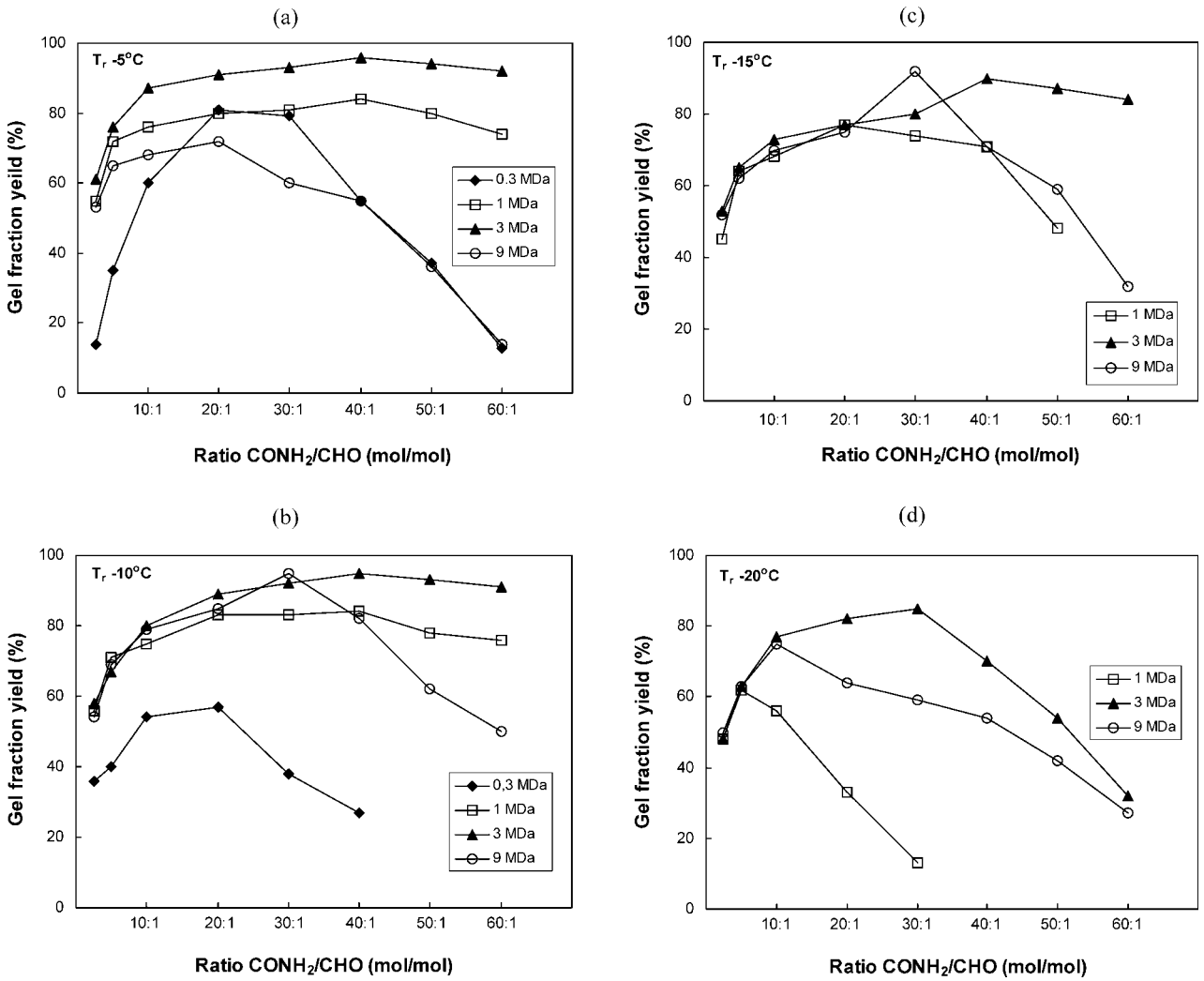

Figure 3 Dependence of the gel fraction yield on the $CONH_2$/CHO molar ratio in the initial reaction solution for cryo-PAAG samples synthesized at negative temperatures: (a) −5, (b) −10, (c) −15, and (d) −20°C.

aqueous medium at −8°C, and the biggest gel fraction yield was found for the precursor of the highest molecular weight.[24] In this work, one can see (Figs. 2 and 3) that for the formation of both PAAGs and cryo-PAAGs, there was no such direct correlation between the efficiency of the process (gel fraction yield) and the PAAm molecular weight. Mainly, the best results were obtained when, for the preparation of gels and cryogels, we employed PAAm with $M_\eta \sim 3$ MDa rather than a polymer of a higher molecular weight, namely, PAAm with $M_\eta \sim 9$ MDa. In other words, just like the use of 1-MDa PAAm, the use of 9-MDa PAAm mainly gave rise to a worse gel fraction yield in comparison with the implementation of 3-MDa PAAm over the studied range of $CONH_2$/CHO ratios. We believe that this finding can be explained by the competition of such favorable gelling factors as the entanglement of macromolecular chains, which, at an equal polymer concentration, increases with an increase in the chain length (molecular weight), and of such unfavorable factors as the polymer's molecular-weight-dependent progressive increase in the viscosity of the reaction medium (ULMP in the case of cryotropic gelation). Therefore, apparently for every particular cryogenically gelling system, there is a certain optimum combination of the precursor's molecular weight characteristics, its concentration, and the polymer/cross-agent ratio capable of resulting in as high as possible efficiency of the gel formation process.

Yet another effect that needs to be discussed here is the bell-like character of the dependences in Figures 2 and 3. In the previous study,[8] we already observed similarly shaped dependences upon the preparation of PAAGs and cryo-PAAGs from PAAm with $M_\eta \sim 3$ MDa, and this was attributed to the kinetic factors of the crosslinking process. This known phenomenon[25–27] takes place at high concentrations of a crosslinking agent when the formation of junction knots of polymeric networks occurs very rapidly, thus significantly

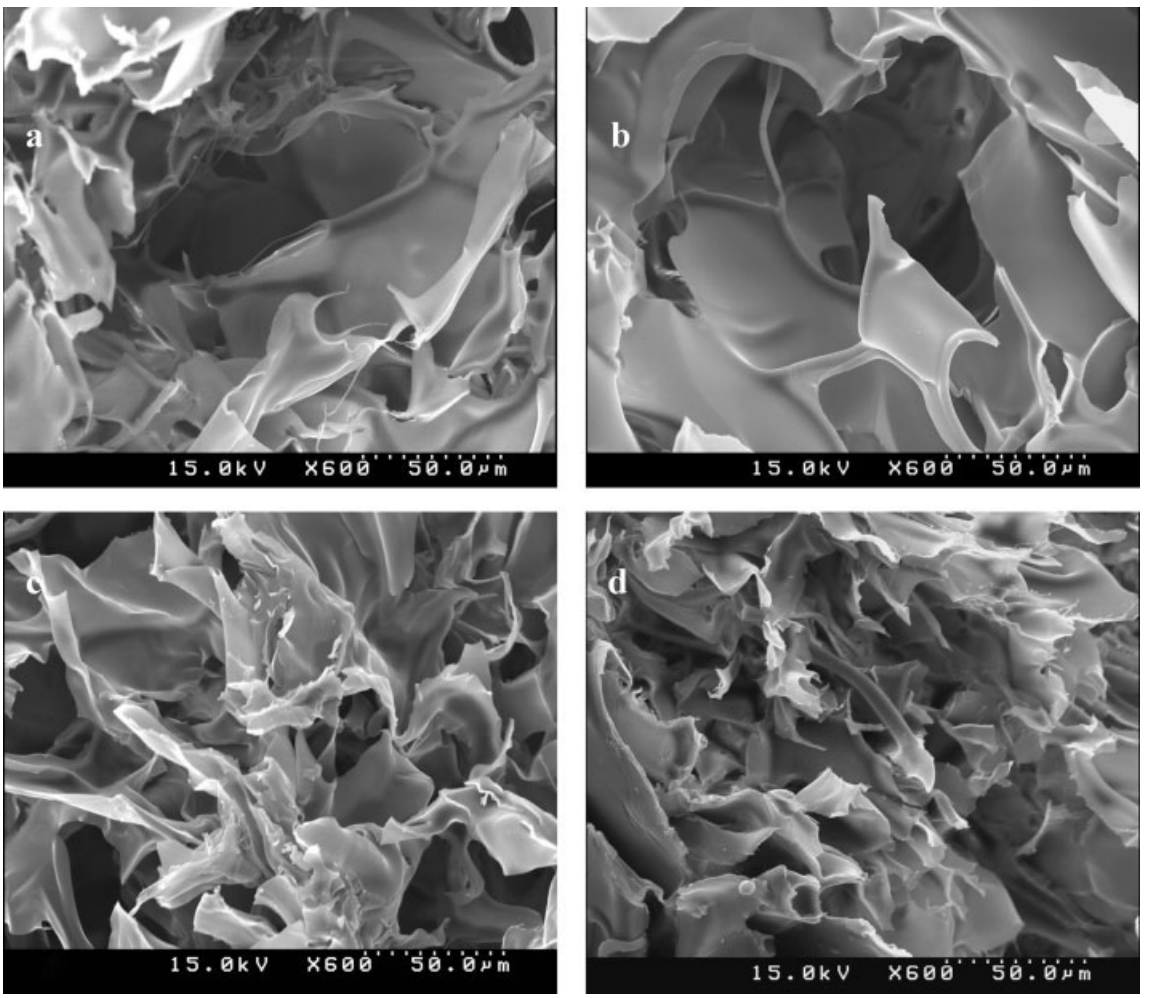

Figure 4 SEM micrographs of cryo-PAAG samples prepared from PAAm with approximate M_η values of (a) 0.3, (b) 1, (c) 3, and (d) 9 MDa at −10°C. The $CONH_2$/CHO ratio was 10 : 1 (mol/mol).

hindering the mobility of the reacting polymer and limiting the realization of optimal possibilities for the crosslinking process.[27] Now the same (qualitatively) curves were found for polymeric precursors of other molecular weights as well. This certainly testifies to the common nature of the effects: with an increase in the concentration of the cross agent, the gel fraction yield initially rises, then reaches a certain limiting value, and further begins to decrease. Such a pattern was observed for all the cases studied in this work when both PAAGs (Fig. 2) and cryo-PAAGs (Fig. 3) were synthesized with PAAm precursors with molecular weights of 0.3–9 MDa.

Morphological features of cryo-PAAGs prepared from PAAm samples of different molecular weights

Figure 4 presents SEM micrographs of cryo-PAAG specimens prepared from PAAm samples of different molecular weights at identical $CONH_2$/CHO molar ratios equal to 10 : 1. The molecular weight of PAAm exerted a certain influence on the morphology of the resulting cryogels. All these cryo-PAAGs possessed a macroporous texture (closed in its character) inherent in similar spongelike cryogels,[1] and the observed differences were evidently connected to the molecular weight of the polymeric precursor employed in every particular case.

Taking in to account some changes in the structure of the gel materials caused by the procedures used in the sample preparation for the SEM studies,[12] we could measure (for the sake of qualitative comparison) the pore size in the micrographs of respective cryo-PAAGs formed from PAAm with M_η values of 0.3 [Fig. 4(a)], 1 [Fig. 4(b)], 3 [Fig. 4(c)] and 9 MDa [Fig. 4(d)].

One can see that with an increase in PAAm's molecular weight, the pore size of the gel samples diminished systematically from about 100–150 μm in the cryogel prepared from the 0.3-MDa polymeric precursor to about 20–50 μm in the cryogel prepared

from the 9-MDa precursor. Hence, one can draw the conclusion that the lower the molecular weight is of a precursor (the shorter its polymeric chain), the bigger the pore size is in the resulting cryogel, and this proves that all other parameters of the gel formation process are identical. Such a trend can obviously be explained from the viewpoint of unequal amounts of the unbound (freezable) water in equiconcentrated solutions of the same polymer but of different molecular weights. Indeed, the longer the polymeric chains are, the higher the volume is that is occupied by their solvated coils. Thus, in accordance with the well-recognized Mark–Kuhn–Houwink equation ($[\eta] = K$ (Huggins constant) $\times M_\eta^\alpha$), $[\eta]$, which is the volume of a polymer in an infinitely dilute solution, is in a power dependence ($\alpha \leq 1$) on the molecular weight of the polymer. Consequently, a lower amount of free water can be frost-solidified at the same negative temperature in moderately frozen aqueous systems containing a dissolved polymer of a higher molecular weight. Therefore, the lower the amount is of crystallized ice, the smaller the volume is of ice particles (they perform as porogens upon the formation of cryogels[1–7]). In the case of the freeze–thaw-induced formation of cryo-PAAGs from macromolecular precursors, this effect resulted in a decreasing pore size with a simultaneous increase in the number of pores per unit of volume, as seen in a comparison of the micrographs in Figure 4.

In addition, these SEM pictures show the interconnected character of the macropores in such spongy gel materials. This is a characteristic feature of cryogel morphology in general, and the reason for such interconnected porosity lies in the fact that during solvent crystallization each individual ice crystal grows until tight contact with a facet of another crystal, which, after system thawing, results in the interconnected macropores.[1]

Swelling characteristics of cryo-PAAGs synthesized from PAAm samples of different molecular weights

Because of the supermacroporous structure of spongelike cryogels, such as the cryo-PAAGs studied in this work, those upon swelling are capable of absorbing a solvent in two distinct ways: by the solvation of the polymer network and by the solvent soaking through the system of interconnected gross pores because of the capillary forces. The former parameter, that is, the swelling extent of the crosslinked 3D network [$S_{w/w}$ (g of H_2O/g of dry polymer)] is well known to be indicative of the crosslinking density and, to a degree, the network's morphology (e.g., the presence of topological gearings that can somewhat limit the swelling process).[13,14,27] Therefore, we have measured the $S_{w/w}$ values for PAAGs (Fig. 5)

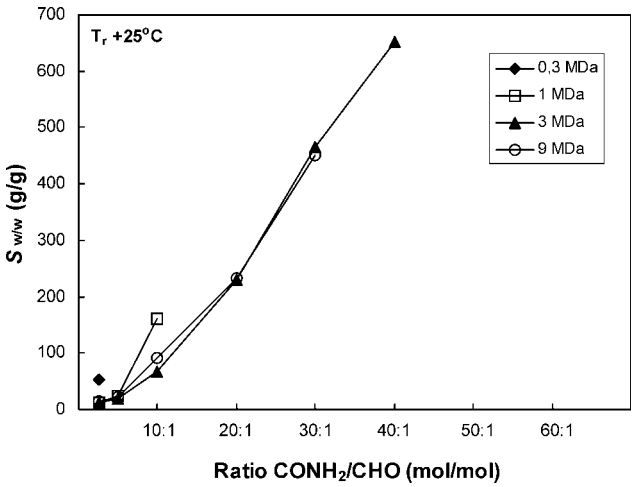

Figure 5 Dependence of $S_{w/w}$ on the $CONH_2$/CHO molar ratio in the initial reaction solution for PAAG samples synthesized at +25°C.

and cryo-PAAGs (Fig. 6) formed from feeds containing PAAm samples of different molecular weights and various GA concentrations.

In the case of gel formation in a liquid nonfrozen system at a positive temperature, the $S_{w/w}$ values for PAAGs were considerably higher (Fig. 5) than the values for respective cryogels (that is, those synthesized from the same initial reagent solutions; Fig. 6). Such differences are typical for the gels and cryogels that are formed in media with very distinct real concentrations of precursors: the gels are formed in solutions containing reagents in amounts assigned by an experimenter, and the cryogels are formed in a concentrated medium of ULMP, in which the real concentrations of the precursors are governed by the temperature of a moderately frozen system.[1,28]

PAAGs prepared at 25°C through the crosslinking of PAAm ($M_\eta \sim 0.3$, 1, 3, or 9 MDa) with GA were found to possess $S_{w/w}$ values ranging from 12 to 650 g/g (Fig. 5), which depended on the initial $CONH_2$/CHO ratio. The gels' swelling capability very strongly increased with decreasing GA content in the initial feed, and such swollen PAAGs in appearance looked transparent and brittle (at a high crosslinking extent) or mechanically weak (at a low crosslinking extent), in the latter case resembling jelly-like matter. At the same time, in cryo-PAAGs, the polymeric framework [the labyrinth-like system (see Fig. 4) comprises the walls of macropores in spongy cryogels] consists of highly concentrated gel matter, which upon swelling absorbs considerably less water, from 3 to 132 g of H_2O/g of dry polymer; this depends on both the initial $CONH_2$/CHO ratio and the reaction temperature [Fig. 6(a–d)].

As for the influence of the PAAm molecular weight on the swelling parameters of cryo-PAAGs prepared from respective polymeric precursors, we

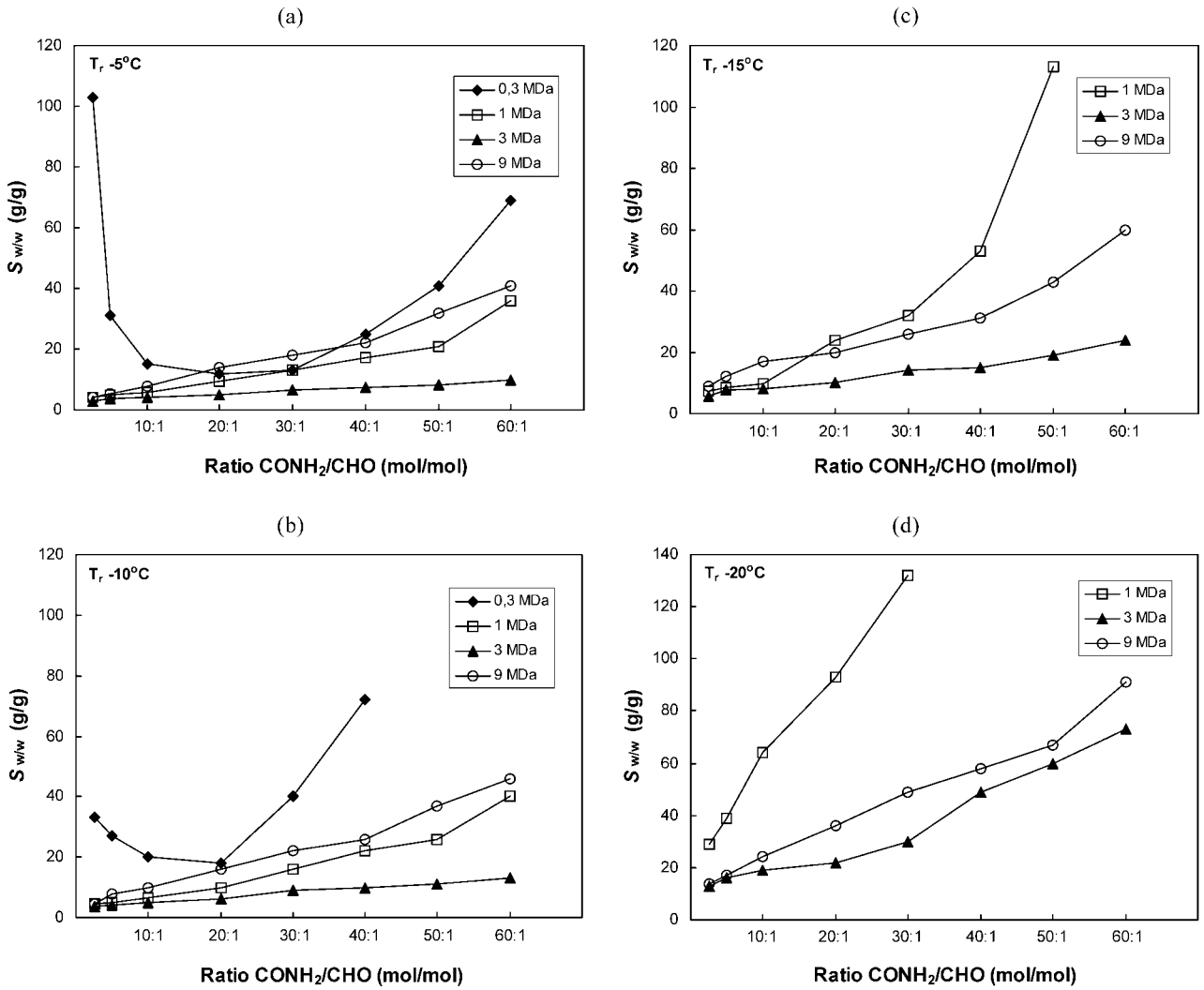

Figure 6 Dependence of $S_{w/w}$ on the $CONH_2/CHO$ molar ratio in the initial reaction solution for cryo-PAAG samples synthesized at different negative temperatures: (a) −5, (b) −10, (c) −15, and (d) −20°C.

observed rather anomalous effects for cryogels synthesized at −5 and −10°C on the basis of PAAm with $M_\eta \sim 0.3$ MDa: the dependences of $S_{w/w}$ on the $CONH_2/CHO$ ratio were of extreme character [Fig. 6(a,b)]. For other polymeric precursors ($M_\eta \sim 1$, 3, or 9 MDa), the same dependences were common; in other words, the $S_{w/w}$ values gradually decreased with the crosslinking extent increasing, that is, with the variation of the $CONH_2/CHO$ ratio from 60 : 1 (the lowest GA initial concentration) to 2.5 : 1 (the highest GA initial concentration).

The reasons for the anomalous character of the dependences in the case of cryogels prepared from PAAm with a molecular weight of 0.3 MDa are not exactly clear. Nonetheless, one can suppose that the left ascending branches of the corresponding curves in Figure 6(a,b) are stipulated by the already mentioned influence of kinetic factors on the crosslinking efficiency. If this is so, the crosslinking reaction starts too fast, commencing from a certain boundary GA concentration and hampering significantly the mobility of reacting species, thus preventing further intermolecular binding. Therefore, the swelling degrees (those depend on the intermolecular crosslinking extent) of the resulting networks begin to grow, despite an increasing amount of the cross agent in the feed. In the case of cryo-PAAG preparation, such an effect was observed only when we used the polymeric precursor with $M_\eta \sim 0.3$ MDa.

A comparison of the $S_{w/w}$ values of cryogels synthesized at the same negative temperature—−5 [Fig. 6(a)], −10 [Fig. 6(b)], −15 [Fig. 6(c)], or −20°C [Fig. 6(d)]—but from high-molecular-weight PAAm samples ($M_\eta \sim 1$, 3, or 9 MDa) showed the lowest swelling capabilities for cryo-PAAGs prepared on the basis of PAAm with $M_\eta \sim 3$ MDa, that is, for cryogels for which the biggest gel fraction yields were also found (Fig. 3). This obviously meant that in the case of such macromolecular precursors, these were the most favorable conditions for the highest efficacy of

PAAm crosslinking by GA, thus giving rise to the formation of cryo-PAAGs with the lowest swelling capabilities of the gel phase in the walls of macropores. Respective cryogels prepared on the basis of PAAm with M_η equal to 1 or 9 MDa possessed higher $S_{w/w}$ values; that is, these samples had poorly crosslinked gel phases of macropore walls.

Finally, we can see that the temperature of cryotropic gelation also influenced the swelling characteristics of the resulting cryo-PAAGs. With the lowering of the reaction temperature from -5 to -20°C, the efficiency of the crosslinking process manifestly decreased because the swelling capabilities of the respective cryogels (i.e., those formed from identical feeds) rose (Fig. 6). In principle, this trend coincided in its character with the previously discussed trend for the influence of the cryogenic reaction temperature on the gel fraction yield (Fig. 3). Hence, we can draw a conclusion about the inhibition effect of temperature lowering (over the range studied) with respect to our particular gelling system. It is noteworthy to point out that very often, when the process of freeze-assisted gel formation is explored, bell-shaped temperature dependences of the parameters characterizing the gelling efficiency are observed because of a competition between the favorable factors (the cryoconcentration of the gel precursors, deceleration of certain side reactions, heat removal for exothermic processes, etc.) and unfavorable factors (high viscosity in ULMP and low thermal mobility of reagents).[1,6] Apparently, in this case, that is, in the case of PAAm crosslinking with GA, a maximum point for the dependence under discussion could lie at a temperature somewhat higher than -5°C but evidently below the melting point of this frozen reacting system.

CONCLUSIONS

Supermacroporous, spongelike cryo-PAAGs were prepared in moderately frozen (-5 to -20°C) aqueous media through the crosslinking reaction of water-soluble linear polymeric precursors of different molecular weights (M_η = 0.3, 1, 3, or 9 MDa) with various concentrations of GA at a pH around 10. The gel formation efficiency (gel fraction yield) and pore size of such cryogels were found to be dependent not only on the gel formation temperature but also on the precursor's molecular weight. However, if the size of the gross pores in these cryogels decreased monotonically with an increase in the molecular weight of the initial PAAm, a bell-shaped dependence of the gel fraction yield on the precursor's molecular weight was observed. The highest efficiency of such cryotropic gel formation was shown to be inherent in the case when PAAm with M_η equal to about 3 MDa was employed. In addition, quite spongy cryogels had the lowest swelling capacities for their gel phase (i.e., their networks were more strongly crosslinked) in comparison with other cryogels formed on the basis of polymeric precursors of both lower (0.3 or 1 MDa) and higher (9 MDa) molecular weights. This finding demonstrates the significance of the true choice of the molecular weight characteristics of a polymer used as a high-molecular-weight precursor when respective macroporous cryogels are being prepared via precursor crosslinking with a low-molecular-weight cross agent.

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