Preparation and Characterization of Polyacrylamide Cryogels Produced from a High-Molecular-Weight Precursor. I. Influence of the Reaction Temperature and Concentration of the Crosslinking Agent

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ABSTRACT: A new type of cryogel was prepared through a reaction of high-molecular-weight polyacrylamide (viscosity-average molecular weight $\approx 3 \times 10^6$ Da) with glutaraldehyde in a moderately frozen aqueous medium. The influence of the crosslinking agent concentration and temperature of the reaction on the gel fraction yield, swelling characteristics, and morphology of the cryogels was investigated. The dependence of the gel fraction yield on the reaction temperature was bell-shaped. The recognized regularities of the formation of this new type of polyacrylamide cryogel based on a high-molecular-weight precursor were very similar to those observed earlier for polyacrylamide cryogels synthesized through the cryopolymerization of monomeric precursors. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 1470–1475, 2007

Key words: cryogel; polyacrylamide; crosslinking

INTRODUCTION

Chemically linked (covalent) and physically linked (noncovalent) cryogels composed of natural or synthetic polymers are heterophase gel materials whose formation is accomplished in moderately frozen systems, that is, at temperatures not lower than several tens of degrees centigrade below the crystallization point of a neat solvent.[1] The characteristic feature of the texture of cryogels is macroporosity. That is, the size and geometry of the gross pores are dependent on the properties and concentration of the precursors for the cryogels and on the cryostructuring regimes. Studies of cryogels and cryotropic gelation processes are actual because cryogels of various chemical natures (especially hydrogels) represent prospective materials for fields such as biotechnology and medicine.[1–7]

There are two principal ways to synthesize covalent cryogels:

1. Through the branched cryopolymerization of monomeric precursors (subscript *i* is used to denote this type of cryogel).
2. Through the chemical crosslinking of high-molecular-weight precursors (subscript *ii* is used to denote this type of cryogel).

Both possibilities have been realized and explored.[1] Among the materials thus prepared, polyacrylamide (PAAm) cryogels (cryoPAAG$_i$) are related to some of the best studied. These supermacroporous, sponge-like gel matrices, which are produced by the free-radical copolymerization of acrylamide (AAm) and *N*,*N*′-methylenebisacrylamide (MBAAm) in frozen aqueous or organic media (e.g., formamide), were first described in the early 1980s,[8,9] and then the peculiarities of the properties, structures, and formation processes of cryogels were carefully researched.[10–13] The main regularities found were recently also confirmed with respect to both cryoPAAG$_i$ and those gels containing certain additional monomer units.[14–20] It is reasonable to point out the promising applied potential of such cryoPAAG$_i$-based gel matrices, such as continuous chromatographic beds for the isolation and purification of high-molecular-weight solutes[21] or bioparticles[22] and scaffolds for animal cell culturing.[23]

At the same time, PAAm cryogels produced from polymeric precursors (cryoPAAG$_{ii}$) were not known

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yet, although such materials could be of significant interest because, when chemically crosslinking macromolecules (in this case, linear PAAm prepared in advance) in a solution, one could reach a more uniform distribution of junction knots in the gel matter in comparison with gels and cryogels produced through the copolymerization process. Thus, it has been shown that AAm and MBAAm at different stages of cryopolymerization reactions are inserted into the gel network nonproportionally to the ratio in the feed, and this is due to the marked differences in the solubility of the comonomers (MBAAm is more hydrophobic than AAm) at reduced temperatures.[13] Furthermore, in the case of cryoPAAG$_{ii}$, there are more possibilities for varying the crosslinking degree, especially at high crosslinking extents, and one can, if it is necessary, trace the influence of the molecular weight of the polymeric precursor on the properties of the final cryogels.

This work describes the preparation of cryoPAAG$_{ii}$ with a high-molecular-weight precursor (a 3-MDa PAAm formed as a result of AAm cryopolymerization[24]) and a low-molecular-weight crosslinking agent [glutaraldehyde (GA) in this case]. The goal of this study was to elucidate the influence of the conditions of the cryogenic treatment on the osmotic properties and morphology of cryoPAAG$_{ii}$ and on the efficiency of cryotropic gelation (yield of a gel fraction).

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 GA was obtained from Fluka (Buchs, Switzerland).

Synthesis of linear PAAm

AAm was dissolved in doubly distilled water and then deaerated by argon bubbling (10 min). This solution was further chilled in an ice bath to 1–2°C. The required amounts of TMEDA and APS were added, and then the reaction mixture was frozen in a chamber of an NCB-3100 cryostat (Eyela, Tokyo, Japan) and kept at −14°C for 24 h (this temperature has been shown to allow PAAm to be obtained with a high polymerization degree).[24] The frozen sample was thawed at room temperature, and the polymer that formed was separated from the low-molecular-weight admixtures by dialysis through the benzoylated cellulose membrane (Sigma, St. Louis, MO; cutoff limit $\approx 2 \times 10^3$ Da) 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]) of a PAAm specimen in a 1*M* $NaNO_3$ solution was determined with an Ubbelohde capillary viscometer (Daihan Scientific, Seoul, Korea) at 30°C. The viscosity-average molecular weight (M_η) of the polymer was calculated with the following equation:[25]

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

Preparation of the PAAm gels and cryogels

PAAm with $M_\eta \approx 3 \times 10^6$ Da was used to prepare PAAm gels (PAAG$_{ii}$) and cryoPAAG$_{ii}$. A fixed amount of TMEDA (aprotic base) was added to a 2% water solution of the polymer to adjust the pH value up to 10. The resulting solution was chilled to 1–2°C if the crosslinking reaction was carried out at negative temperatures. The required amount of GA was added to the PAAm solution, and the mixture was stirred for 30 s. Then, the samples were placed in the chamber of the cryostat or a CW-05G thermostat (Jeio Tech, Daejeon, Korea) for 24 h. Because the reaction system did not crystallize at −5°C on account of supercooling effects, the specimens, before their incubation at this temperature, were placed in liquid nitrogen for 15 s to crystallize most of the solvent. The thus frozen reaction system was then transferred to the cryostat with a coolant temperature equal to −5°C. The cryogels that formed 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

The measurement of the swelling degree of the gel phase was carried out with a known procedure.[10] The swollen specimen was placed on a porous glass filter, closed, and squeezed under a vacuum for 5 min out of the unbound water. For this time, the unbound water moved away from the specimen. Furthermore, the wet preparation was weighed and then dried in a vacuum-drying oven to a constant weight. Because the swollen PAAG$_{ii}$ did not contain unbound water on account of the microporous structure, it was not necessary to squeeze out such samples with the aforementioned manipulations. The amount of gel-bound water, that is, the swelling degree of the polymer phase [$S_{w/w}$ (g of H_2O/g of dry gel)], was calculated with the following formula:

$$S_{w/w} = (m_{ws} - m_{ds})/m_{ds}$$

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

Microscopic investigations

The morphology of the cryogels was studied with an S-4100 scanning electron microscope (Hitachi, Japan). The critical point drying (CPD) technique was employed to prepare the samples for scanning electron microscopy (SEM) according to the conditions described in the literature.[26] The chemical fixing of the cryogel specimens was carried out as follows: the samples were placed in a 2.5% solution of GA in a 0.2*M* sodium phosphate buffer (pH 7.5) for 3 h at 25°C. Then, the specimens were rinsed with water and placed into aqueous solutions of ethanol with concentrations of 30, 50, 70, 80, 90, and 95% for 15 min and then into isoamyl acetate for 20 min (two times). Furthermore, these samples were transferred to an HCP-2 CPD device (Hitachi, Tokyo, Japan), and after drying, they were coated with a silver paste.

RESULTS AND DISCUSSION

It is well known that the formation of a gel phase in polymeric systems such as heterophase cryogels takes place in a so-called unfrozen liquid microphase,[1] which represents a small volume of a solution; it still occurs in the liquid state within the macroscopically frost-bound system, and the solutes are concentrated in such a microphase. Because of the phase inhomogeneity and cryoconcentration of the reagents, chemical reactions not only continue without termination but can even proceed faster in a certain range of negative temperatures.[27] In this connection, it was of interest not only to prepare PAAm cryogels from a macromolecular precursor, that is, to produce cryoPAAG$_{ii}$ and to study the properties of this new type of PAAm-based cryogel, but also to compare them with well-studied cryoPAAG$_i$.

Figure 1 shows a scheme of the crosslinking reaction used by us for the synthesis of the gels and cryogels. The scheme is based on the hydroxyalkylation reaction[28] of amides with aldehydes in alkaline media.

The PAAG$_{ii}$ samples, formed as a result of PAAm crosslinking with GA, were typical homophase hydrogels. In the swollen state, they were transparent and rather fragile. The higher the crosslinking degree was, the more fragile the gels were. The whole volume of the solvent (water) in these gels was the solvate liquid, and it could not be removed through squeezing without disruption of the gel samples. Cryogels that formed from the same initial solutions were heterophase, spongy, nontransparent materials, from which, unbound by a gel phase, the capillary solvent could be separated easily at low mechanical compression without the destruction of the specimens. Therefore, it was convenient, upon the rinsing of the samples, to squeeze unbound

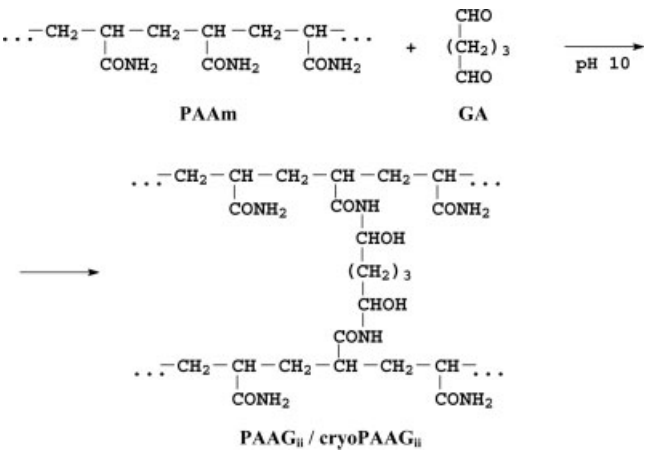

Figure 1 Scheme of the crosslinking of PAAm by GA in solution.

water from the swollen cryogels under a vacuum and then, after drying and weighing, to determine the yield of the gel fraction.

Table I summarizes the gel fraction yields for the samples of PAAG$_{ii}$ prepared at 25°C and for the samples of cryoPAAG$_{ii}$ synthesized at different negative temperatures with various ratios of PAAm and GA (expressed as the molar ratio of pendant amide functions in the polymeric precursor to the aldehyde groups of the crosslinking agent). First, one can see that over the range of $CONH_2$/CHO ratios from 5 : 1 to 40 : 1 (mol/mol), the yields in the case of PAAG$_{ii}$ were considerably lower than those for cryoPAAG$_{ii}$. At the ratio of 2.5 : 1, the yields were very close, and at low concentrations of GA in the feed, no formation of PAAG$_{ii}$ at +25°C occurred at all, whereas from the same initial reagent solutions, the cryogels were obtained with a sufficiently high gel fraction yield. Such an effect is rather well documented for both types of covalent cryogels, that is, those formed from monomeric precursors and those produced by the chemical crosslinking of polymeric precursors.[9–13,30–32] The main reason for this shift in the critical concentration of gelation is ascribed to the aforementioned cryoconcentrating phenomenon, in which the formation of a three-dimensional (3D) polymeric network in cryogels proceeds in a more concentrated medium (unfrozen liquid microphase) in comparison with the initial solution.[1]

The next feature of the data in Table I is a bell-like dependence of the yield values on the initial ratio of the gel and cryogel precursors, that is, PAAm and GA. There is a maximum point for each synthesis temperature: at the molar ratio of $CONH_2$/CHO = 5 : 1 for the gel formation at +25°C and in the vicinity of $CONH_2$/CHO ratios of 30 : 1–40 : 1 (mol/mol) for the cryotropic gelation processes from −5 to −20°C. A similarly extreme type of dependence is known for the formation of covalent gels through the chemi-

TABLE I
Yields of the Gel Fractions for $PAAG_{ii}$ and $CryoPAAG_{ii}$ Samples Synthesized at Various Temperatures

$CONH_2$/CHO ratio (mol/mol)	Gel fraction yield (%)				
	+25 (°C)	−5 (°C)	−10 (°C)	−15 (°C)	−20 (°C)
2.5 : 1	53 ± 2	61 ± 2	58 ± 1	53 ± 2	48 ± 2
5 : 1	68 ± 2	76 ± 1	67 ± 2	65 ± 2	63 ± 1
10 : 1	57 ± 1	87 ± 1	80 ± 2	73 ± 1	77 ± 2
20 : 1	32 ± 1	91 ± 1	89 ± 1	77 ± 1	82 ± 1
30 : 1	26 ± 2	93 ± 1	92 ± 1	80 ± 1	85 ± 1
40 : 1	19 ± 1	96 ± 1	95 ± 1	90 ± 1	70 ± 2
50 : 1	—[a]	94 ± 1	93 ± 1	87 ± 1	54 ± 1
60 : 1	—[a]	92 ± 1	91 ± 1	84 ± 1	32 ± 1

[a] No gel formation at all.

cal crosslinking of macromolecular precursors in a solution[33] and has also been observed for cryogels.[30,31] A certain decrease in the gel fraction yield at a high concentration of the added crosslinking agent might be explained by the kinetic factors of bounding events. The formation of junction knots of the 3D network appearing occurs in this case very rapidly, thus considerably inhibiting the mobility of the polymer chains and their segments and pendant reactive groups; this, in turn, does not allow optimal possibilities for crosslinking reactions to be realized.[33] Without a doubt, in the case of the formation of cryogels, when the reagent concentrations in the unfrozen liquid microphase are so high, the same inhibiting mechanisms should take place as well.

Finally, Table I shows that at the $CONH_2$/CHO molar ratios of 60 : 1, 50 : 1, and 40 : 1 a bell-shaped dependence of the gel fraction yield on the reaction temperature can be observed. The explanation of such an extreme temperature dependences is usually accomplished on the basis of the competition between the oppositely directed trends.[1,27,30,31] This is the already mentioned freeze-induced increase in the solute concentrations favoring the acceleration of chemical reactions and increasing the yield of the final products. On the other hand, the reaction efficiency diminishes with the decrease in the temperature (decreased thermal mobility of the reactants) and with a progressive increase in the viscosity of the reaction medium as the gel is formed. Rather obviously, within the given range of $CONH_2$/CHO ratios, we dealt with such competition. However, at a higher fraction of GA in the feed ($CONH_2$/CHO = 20 : 1–2.5 : 1 mol/mol), the dependences were somewhat more complicated, thus testifying to the simultaneous influence of some additional factors on the results of the crosslinking reaction in the frozen systems. The exact identification of these additional factors requires further study.

Figure 2 presents SEM micrographs of $cryoPAAG_{ii}$ specimens obtained at various negative temperatures through the crosslinking of PAAm with GA ($CONH_2$/CHO molar ratio = 10 : 1). It is obvious that the gel-formation temperature exerts a significant influence on the morphology of the cryogels. With the temperature decreasing, the rate of solvent crystallization increases, more crystallization germs arise, and thus the generated crystals have smaller dimensions.[29] The polycrystals of the frozen solvent (in this case, ice crystals) act as porogens in the forming cryogels (here, $cryoPAAG_{ii}$). Thus, the reduction of the size of the porogen particles caused by a decrease in the freezing temperature should result in the diminution of the size of the macropores in the respective cryogels, and this was observed in SEM investigations of $cryoPAAG_{ii}$ (Fig. 2). The pore diameter varied from 30–60 μm in the cryogel formed at −5°C, as shown in Figure 2(a), to 10–20 μm in the cryogel formed at −20°C, as shown in Figure 2(d). Moreover, the SEM pictures demonstrate the interconnected character of such macropores in the cryogels. This is a characteristic feature of cryogels' morphology, in general, whereas with the freezing of the initial solution of the cryogel precursors (PAAm and GA), each crystal of the solvent (porogen) grows from the vessel's periphery toward the center (if no directional freezing is implemented) until the crystal comes into contact with the facet of another one,[1,5–7] and this, in turn, after the thawing of the specimen results in a system of interconnected macropores in the cryogel formed.

The SEM photographs illustrating the texture of $cryoPAAG_{ii}$ prepared from the high-molecular-weight precursor (Fig. 2) turned out to be very similar to the SEM pictures of $cryoPAAG_i$ formed at respective negative temperatures via the cryopolymerization of the monomeric precursors.[9–13] Such a similarity was observed with respect to the size of the macropores (tens of micrometers), the dependence of the pore diameter on the freezing temperature, and the character of the macroporosity architecture. This evidently means that the general mecha-

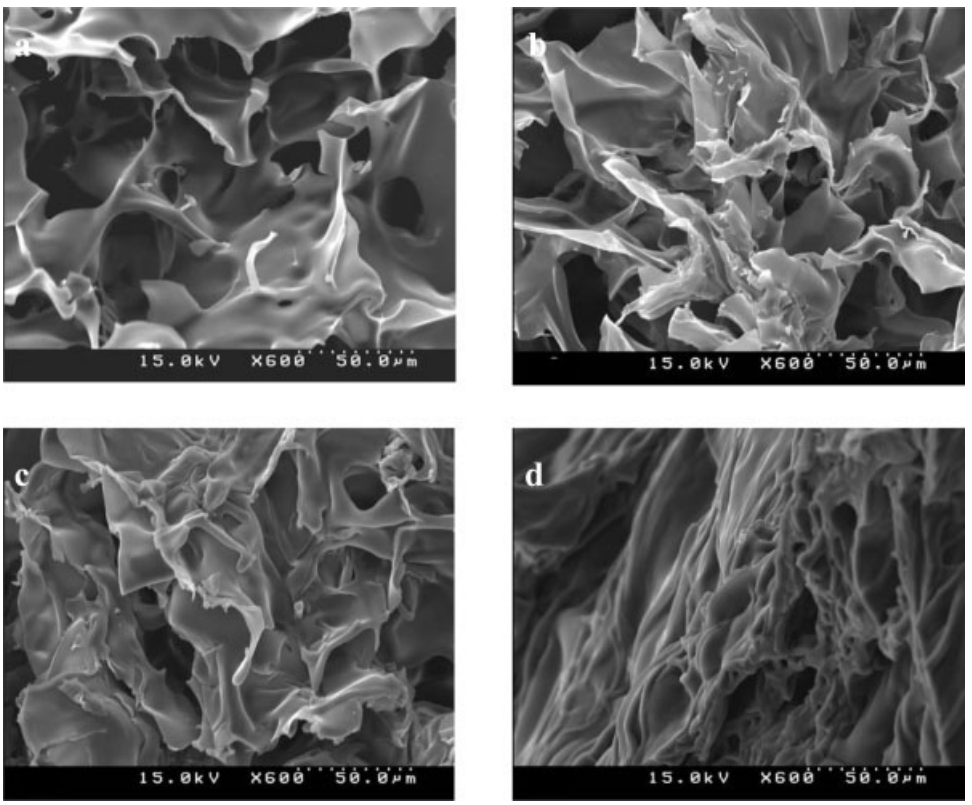

Figure 2 SEM micrographs of cryoPAAG$_{ii}$ samples prepared at (a) −5, (b) −10, (c) −15, and (d) −20°C. The $CONH_2$/CHO ratio was 10 : 1 (mol/mol).

nisms for the formation of macroporous structures in these two types of polymeric cryogels are virtually analogous.

Taking into account the spongelike morphology of PAAm cryogels, we find that the total volume of a solvent absorbed by such a gel material upon swelling consists of two entities: an unbound capillary liquid and a solvate one tightly bound by the network of the polymer phase. The second constituent, $S_{w/w}$ (see the Experimental section), can be considered an indicator of the crosslinking extent and thus can testify to the efficiency of the crosslinking reaction at various temperatures. The values of $S_{w/w}$ were measured, and Table II collects the obtained data.

For both PAAG$_{ii}$ and cryoPAAG$_{ii}$ samples prepared and examined in this work, one can see a clear regularity: the lower the portion of GA added to the initial feed, the higher the swelling degree of the respective (i.e., synthesized at equal temperatures) gels and cryogels. The $S_{w/w}$ values for gels were considerably higher (up to 2 orders of magnitude) than the values for cryogels. Certainly, this reflected the phenomenon of an increase in the solute concentration when the cryotropic gelation took place in a concentrated (with respect to PAAm and GA) unfrozen liquid microphase, and this gave rise to the formation of a dense, weakly swelling 3D network. For instance, cryoPAAG$_{ii}$ prepared at −10°C from the

TABLE II
$S_{w/w}$ Values for the Gel Fractions of PAAG$_{ii}$ and CryoPAAG$_{ii}$ Samples Synthesized at Various Temperatures

$CONH_2$/CHO ratio (mol/mol)	$S_{w/w}$ (g of H_2O/g of the dry polymer)				
	+25 (°C)	−5 (°C)	−10 (°C)	−15 (°C)	−20 (°C)
2.5 : 1	13 ± 1	3.0 ± 0.1	3.7 ± 0.1	5.5 ± 0.2	13 ± 1
5 : 1	18 ± 3	3.5 ± 0.2	4.3 ± 0.2	7.5 ± 0.3	16 ± 1
10 : 1	66 ± 7	4.1 ± 0.3	5.0 ± 0.1	8.0 ± 0.2	19 ± 2
20 : 1	230 ± 14	5.0 ± 0.5	6.2 ± 0.5	10 ± 1	22 ± 1
30 : 1	465 ± 19	6.4 ± 0.3	9.0 ± 0.7	14 ± 1	30 ± 2
40 : 1	653 ± 27	7.3 ± 0.2	10 ± 1	15 ± 1	49 ± 2
50 : 1	—	8.2 ± 0.5	11 ± 1	19 ± 1	60 ± 4
60 : 1	—	9.6 ± 0.6	13 ± 1	24 ± 1	73 ± 4

feed with the lowest amount of GA ($CONH_2$/CHO molar ratio = 60:1) turned out to possess the same swelling parameter (~13 g of H_2O/g of the dry polymer) as $PAAG_{ii}$ formed at +25°C from the solution with the highest GA concentration ($CONH_2$/CHO molar ratio = 2.5 : 1). Such differences between the $S_{w/w}$ values for gels and cryogels are also characteristic features for $PAAG_i$ and $cryoPAAG_i$ prepared from monomeric precursors.[9,10,12,13]

As for the temperature dependence of the swelling degree of the cryogels, over the temperature range studied and at all GA concentrations used, we did not observe any anomalies. In all cases, $S_{w/w}$ for spongy cryogels increased monotonically with a decrease in the cryotropic gelation temperature, thus testifying to the certain fall of the crosslinking efficiency with decreasing temperature.

CONCLUSIONS

A new type of PAAm cryogel was prepared on the basis of a high-molecular-weight precursor, PAAm, and a low-molecular-weight crosslinking agent, GA; the gel-formation process was performed in moderately frozen (−5 to −20°C) aqueous media. The gel materials thus synthesized had a spongelike, supermacroporous morphology and were similar in this respect to the known PAAm cryogels produced via the cryogenic copolymerization of AAm and MBAAm. The yield of the gel fraction, the osmotic characteristics (swelling behavior) of the novel cryogels, and the architecture of their macropores depended on the concentration of the crosslinking reagent and on the temperature of gel formation.

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