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STUDY OF CRYOSTRUCTURIZATION OF POLYMER SYSTEMS—X. ^{1}H- AND ^{2}H-NMR STUDIES OF THE FORMATION OF CROSSLINKED POLYACRYLAMIDE CRYOGELS

DMITRY G. GUSEV,[1] VLADIMIR I. LOZINSKY[2] and VLADIMIR I. BAKHMUTOV[1]

[1]Institute of Organo-Element Compounds and [2]Institute of Food Substances, Russian Academy of Sciences, 28 Vavilov Str., 117813 Moscow, Russia

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Abstract—Some features of the formation of polyacrylamide cryogels have been studied by means of ^{1}H- and ^{2}H-NMR. These gels were prepared by free radical copolymerization of acrylamide and *N,N′*-methylene-bis-acrylamide in a frozen aqueous medium. Using the spectral changes during polymerization, the cryotropic gelation may be conventionally subdivided into three stages during which the narrow NMR signals due to the low molecular weight components disappear, while the broad signals attributed to the formation of the polymeric compound develop. It was demonstrated that the dynamics of polymerization depend on the freezing procedure, i.e. on the thermal prehistory of a sample.

INTRODUCTION

Previously, freezing of aqueous solutions of acrylamide (AA) and *N,N′*-methylene-bis-acrylamide (MBAA) in the presence of a radical polymerization initiator [a mixture of ammonium persulphate (ASP) and *N,N,N′,N′*-tetramethylethylenediamine (TMED)] was shown to cause the formation of cryogels of cross-linked polyacrylamide (cryoPAAG) [1–5]. These cryogels markedly differ from traditional polyacrylamide gels (PAAG) prepared in liquid solution, which are microporous, transparent and rubbery. CryoPAAG have a turbid appearance and macroporous structure since the formation of the polymer network takes place within the unfrozen zones of the reaction system, whereas the pores are formed by ice polycrystals [2, 4]. These unfrozen zones were called the liquid microphase [6–8].

The kinetics of polymerization in solution at higher temperature and in the frozen system also differ significantly. At low conversions, the temperature dependence of cryoPAAG formation rate is quite unusual, showing a maximum at *ca* $-20°$ [4]. Further, the structure and kinetics of cryoPAAG formation significantly depend on the freezing procedure [5], i.e. they are determined by the thermal prehistory of a sample. All available data demonstrate that cryopolymerization of the AA–MBAA mixture is a complex process, in some respects having an unclear nature.

Previous approaches to the study of cryoPAAG formation included thawing and subsequent investigation of defrosted samples. In this work, we attempted an *in situ* study of cryotropic gelation using high resolution NMR. NMR spectroscopy has already been successfully applied to study PAAG formation in solution [9] and allows one to follow the course of reaction and determine the composition of the liquid microphase in the frozen system [10].

EXPERIMENTAL PROCEDURES

The following reagents were used without purification: acrylamide (AA) (Sigma, U.S.A.); *N,N′*-methylene-bis-acrylamide (MBAA), *N,N,N′,N′*-tetramethylethylenediamine (TMED) and ammonium persulphate (ASP) (Serva, Germany); D_2O (99.8%, Isotop, Russia).

Linear polyacrylamide (PAA) with a viscosity-average molecular weight of 440 kDa was synthesized by polymerization of 3% AA aqueous solution at 293 K. ASP/TMED system was used as initiator. The polymer was precipitated from the reaction mixture with excess methanol, washed with methanol, and dried *in vacuo* (0.1 mm Hg) at 313 K to constant weight.

The reaction solution for cryoPAAG synthesis was prepared in D_2O as described elsewhere [2, 4, 5]. Its composition was: total comonomers content—3% ([AA]:[MBAA] = 30:1, mol/mol), TMED (0.064%) and ASP (0.025%). In all cases, ASP was added directly prior to freezing the reaction mixture.

The ^{1}H- and ^{2}H-NMR data for each investigated temperature were obtained on separately prepared samples using the following freezing procedures:

(A) polytetrafluoroethylene tube of 9 mm o.d. with 1.5 ml of the solution under investigation was placed in the spectrometer probe-head pre-cooled to the desired temperature;

(B) the same tube was placed in liquid N_2 for 30 min, and then transferred to the probe-head.

The ^{1}H- and ^{2}H-NMR spectra were obtained with Bruker CXP-200 or WP-200 SY spectrometers with operating frequencies of 200 and 30.7 MHz for ^{1}H and ^{2}H, respectively.

The relative amounts of unfrozen water and the dissolved substances were estimated by comparative integration of the NMR signals obtained under identical conditions, before and after the freezing, using the spectrometer software in the absolute intensity mode (AI = 1).

For convenience, ΔT value ($\Delta T = T_r - T_0$) is used throughout this paper, where T_r is the temperature of reaction (the temperature in the spectrometer probe-head), and T_0 is the D_2O crystallization temperature (276.8 K [11]).

RESULTS AND DISCUSSION

1. NMR characteristics of liquid and frozen D_2O–AA–MBAA–TMED systems

In the absence of ASP, there is no polymerization in the solution of a mixture of AA–MBAA–TMED and this solution can be studied at any temperature. Consequently, the changes in NMR spectra appearing when ASP is added may be considered to result from cryoPAAG formation.

At 293 K ($\Delta T = +16$ K), the ^{1}H-NMR spectrum of the AA–MBAA–TMED D_2O-solution [Fig. 1(A)] shows TMED signals [δ 2.28(CH_2), 2.01(CH_3)] and resonances of the vinyl protons of comonomers [δ 6.15, 6.03(CH_2), 5.60(CH)] with integral intensities corresponding to the ratio [AA + MBAA]:[TMED] = 145:1. Similar signals are observed [Fig. 1(B)] in the spectra of the frozen system at $\Delta T = -10 \cdots -25$ K. However, in the latter case there is significant line broadening and only averaged TMED protons resonance is detected.

High resolution NMR spectra are known to be observed when the sample molecules are not restrained in their rotational motion, i.e. generally when they are in the liquid phase. Hence, the observation of the resolved resonances in Fig. 1(B) can be regarded as direct evidence for the existence of the unfrozen (liquid) microphase within the frozen AA–MBAA–TMED D_2O-solution. A comparison of the ^{1}H-NMR integral intensities from the spectrum of the unfrozen solution with those of the frozen samples shows that only 3–6% of the initially dissolved comonomers are detected at $\Delta T = -10 \cdots -25$ K. The ratio [AA + MBAA]:[TMED], equal to 145:1 at $\Delta T = +16$ K, also changes considerably after freezing, reducing to 8:1 and 4.4:1 at $\Delta T = -10$ and -25 K, respectively. Further experiments show that, in the ^{1}H-NMR spectra of the frozen solution of a AA–MBAA mixture without TMED, no signals are detected. At the same time, the signals due to TMED, like those shown in Fig. 1, are observed in the spectra of a TMED solution after freezing. Hence, in the system under investigation, it is TMED that forms the liquid microphase with water, whereas the molecules of comonomers are dissolved in it.

The ^{2}H-NMR data on the temperature dependence of the amount of D_2O in the liquid microphase of the frozen AA–MBAA–TMED solution are given in Table 1. Comparison of the D_2O quantity with that of comonomers in the liquid microphase suggests that at $\Delta T = -10$ K and $-15 \cdots -25$ K the ratio [AA + MBAA]:[D_2O] is respectively 2.5 and 9–11 times larger than that before freezing. Thus, direct evidence is obtained for concentrating of reagents in the unfrozen zones of the frozen reaction mixture. Such a concentrating effect was previously suggested

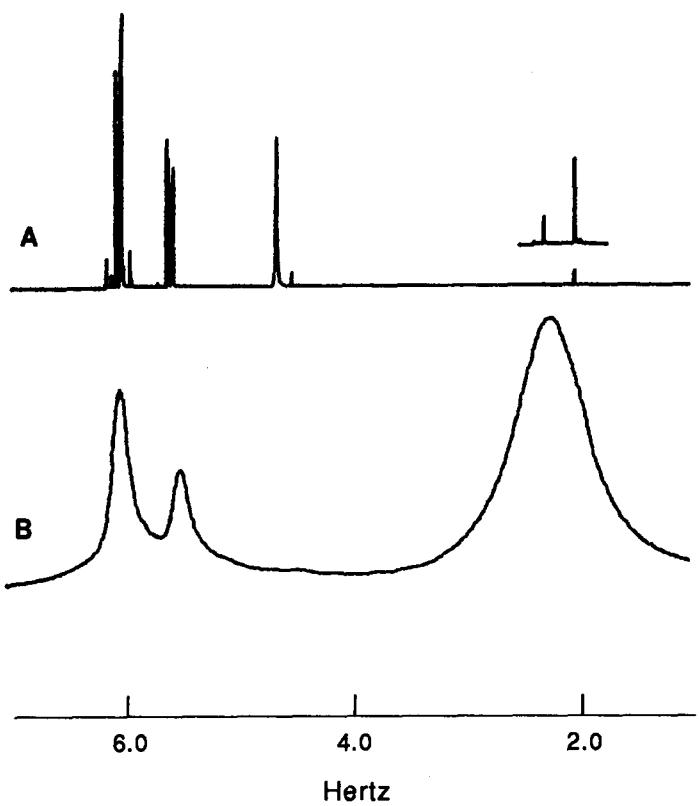

Fig. 1. ^{1}H-NMR spectra of the AA–MBAA–TMED 3%-solution in D_2O at ΔT of +16 K (A) and −15 K (B).

Table 1. ^{2}H-NMR line width and percentage of unfrozen water in frozen AA–MBAA–TMED and PAA solutions (3 wt%) in D_2O

ΔT (K)	Percentage of unfrozen water*		Width (Hz)	
	(AA–MBAA–TMED)	(PAA)	(AA–MBAA–TMED)	(PAA)
−10	2.6	2.6	70	310
−15	0.45	1.7	170	1640
−20	0.28	1.7	260	1900
−25	0.19	1.6	350	2400

*This value was determined from the ratio of integral intensities of D_2O signals at indicated temperatures and at 277 K, respectively. The error is <15%.

on the basis of indirect evidence [2, 3, 5, 6]. Moreover, it appears reasonable that it is because of this concentrating that the degree of swelling of cryoPAAG prepared from a rather dilute AA–MBAA solution is equal to that of PAAG prepared from reaction mixtures containing 20–40 wt% of comonomers.

It seems interesting to compare the ^{2}H-NMR spectra of frozen D_2O–AA–MBAA–TMED and D_2O–PAA systems because the solution of linear polyacrylamide can be considered as a simplified model for a sample where the polymerization process has completed. Table 1 shows that at $\Delta T = -15 \cdots -25$ K the amount of unfrozen water in the D_2O–PAA system is between 4 and 8 times that of the frozen solution of comonomers with TMED. Further, there is an abrupt reduction of the quantity of unfrozen water in the frozen solution of AA–MBAA–TMED mixture below $\Delta T = -10$ K but this value is markedly less dependent on temperature for frozen PAA solution. The latter observation is well known for polymer solutions. It is commonly associated with the presence of tightly bound unfreezable water retaining significant mobility at temperatures well below the freezing point of the free solvent [12–17].

The ^{2}H-NMR data summarized in Table 2 show that the width of the resonance due to mobile water in the frozen PAA solution is significantly greater than that recorded for the frozen D_2O–AA–MBAA–TMED system. This effect can be related to different viscosities of the liquid microphases in these systems implying that there is difference in molecular mobilities of the components in these microphases.

2. NMR spectral changes during the formation of cryoPAAG

It has been previously shown [5] that both freezing procedures A and B (the latter beginning with placing and keeping the sample in liquid N_2) result in crosslinked polyacrylamide cryogels from aqueous solutions of AA–MBAA–TMED–ASP. However, the properties of the cryoPAAG obtained and the polymerization kinetics differ markedly in the two cases, although any chemical reactions in the system should have stopped at the temperature of liquid N_2, as shown already [5]. In this paper, NMR spectra of D_2O–AA–MBAA–TMED–ASP samples prepared by both methods A and B are examined and discussed.

Figure 2 shows the time dependence of the ^{2}H-NMR spectra of the reaction mixture frozen according to procedure A at $\Delta T = -15$ K. Within the first 20 min, when conversion does not exceed several percent [5], the sharp resonance due to D_2O decreases, as the phase transition from bulk water to ice proceeds. Further, a much broader signal appears, concurrent with disappearance of the sharp signal, and after 1 hr only the broad signal (1420 Hz) is observed, remaining unchanged for the next 2 hr. These data suggest that the sharp resonance arises from the bulk water and water in the liquid microphase being formed when the bulk water crystallizes. The broad signal can be assigned to D_2O molecules tightly bound to the polymer obtained, the simultaneous observation of both signals indicating rather slow exchange processes in the liquid microphase.

For a sample prepared by method B, the ^{2}H-NMR spectra (Fig. 3, $\Delta T = -15$ K) show that a relatively narrow signal appears and increases for 12 min after the sample has been transferred to the probe-head from liquid N_2. Further, this signal decreases in intensity with simultaneous increase in intensity of a new broader resonance (1570 Hz). In 25 min, only this resonance remains.

Thus, distinct stages can be distinguished corresponding to the changes in the ^{2}H-NMR spectra shown in Figs 2 and 3. The first stage (I)

Table 2. Stages I and II total duration and ^{2}H-NMR data obtained during stage III for reaction mixtures frozen by means of methods A and B

ΔT (K)	Stages I and II total duration (min)*		Percentage of unfrozen water†		^{2}H-NMR line width (Hz)‡	
	A	B	A	B	A	B
−10	140	25	2.7	2.7	104	350
−15	60	25	1.7	1.7	1420	1570
−20	70	45	1.6	1.6	2040	1970
−25	>90	>90	1.2	1.2	2400	2400

* ± 15%.
†This value was determined from the ratio of integral intensities of D_2O signals at indicated temperatures and at 277 K, respectively. The error is <15%.
‡ ± 5%.

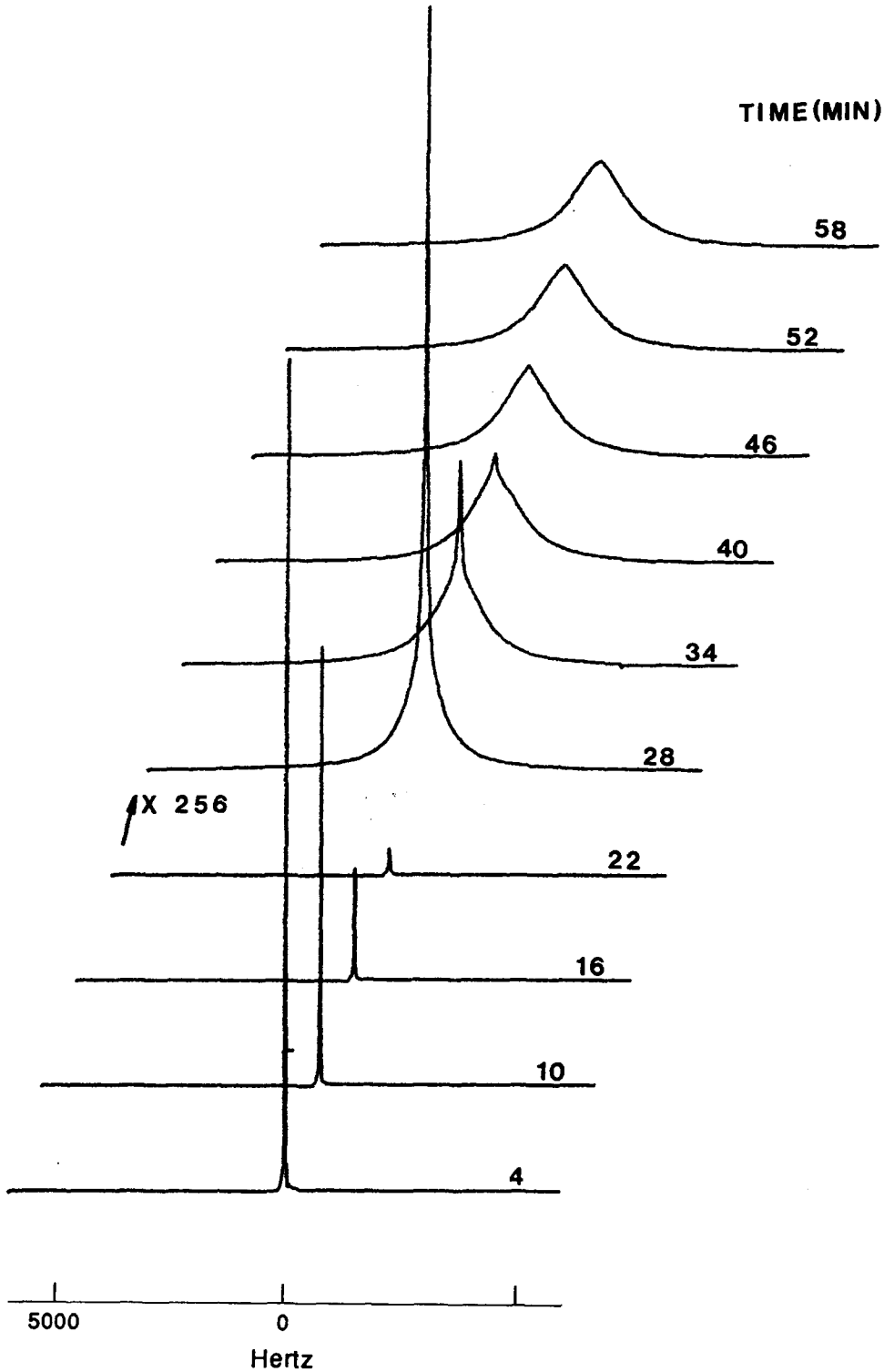

Fig. 2. Time dependence of the ^{2}H-NMR spectra of the D_2O–AA–MBAA–TMED–ASP reaction mixture being frozen at $\Delta T = -15$ K (method A).

involves the phase transitions and, when complete, the spectra become identical to those described above for the reaction mixture in the absence of ASP. The second stage (II) is characterized by the appearance of the broad signal, increasing in intensity simultaneously with the reduction of the former sharp resonance. The third stage (III) corresponds to the observation of a single invariable broad signal due to polymer-bound water.

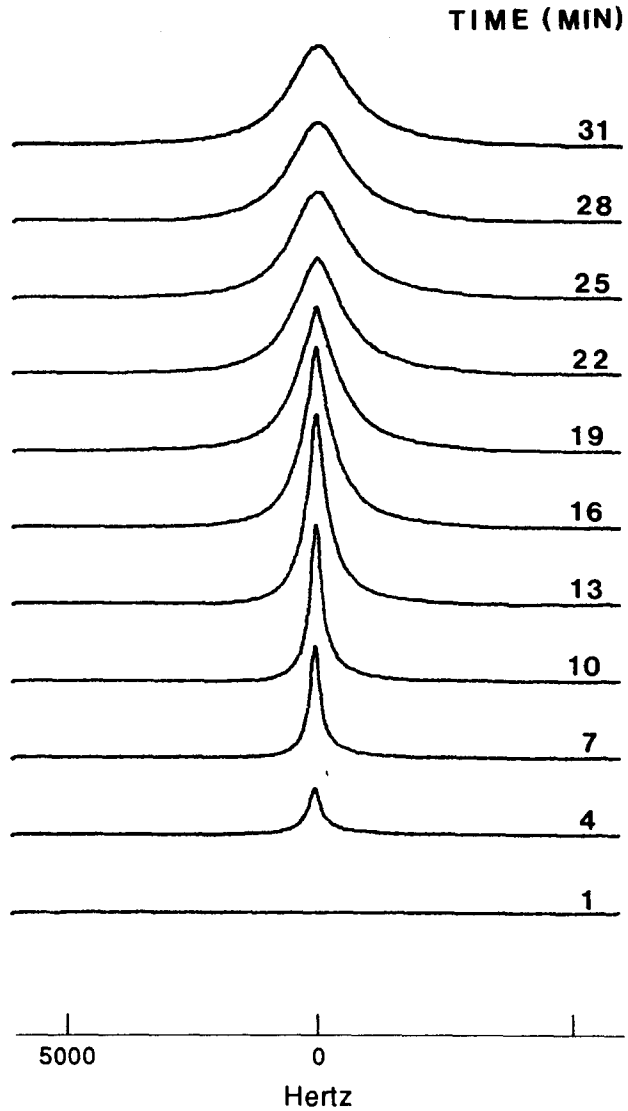

Fig. 3. Time dependence of the ^{2}H-NMR spectra of the D_2O–AA–MBAA–TMED–ASP reaction mixture frozen by method B, $\Delta T = -15$ K.

It should be stressed that cryoPAAG formation is not complete by the end of stage II but proceeds further during stage III. This point is convincingly confirmed by both the published data [5] and the ^{1}H-NMR spectra of the samples defrosted at stage III, showing the presence of the polymer formed along with appreciable quantities of comonomers.

The temperature dependence for the total duration of stages I and II and some NMR data characteristic of the studied system in stage III are reported in Table 2 for both freezing procedures. It is clearly seen that, at $\Delta T = -10 \cdots -20$ K, stage II is completed more rapidly when method B is used. Hence, relating the appearance and the increase of the broad signal due to D_2O with the progress of the polymerization, one can conclude that the initial reaction rate is higher after freezing the samples according to procedure B. Previously [5], the same result was obtained by measuring the yield of gel-fraction. At the same time, the NMR spectra recorded during stage III are practically independent of the freezing procedure and similar to those for the frozen D_2O–PAA system (Table 1).

Figure 4 shows the ^{1}H-NMR spectra of the reaction mixture ($\Delta T = -15$ K, method B) making it possible to follow the changes in the resonances associated with the reagents during polymerization. It is seen that, 6 min after the solution was placed into the spectrometer probe-head, the spectrum was similar to that for frozen solution of comonomers in the absence of ASP [Fig. 1(b)]. Then, the intensities of the signals associated with AA, MBAA and TMED decrease, so that in 20–25 min the ^{1}H-NMR spectra show no sharp resonances. In the similar ^{2}H-NMR experiment (Fig. 3), it took approximately the same time for the changes to be complete. Note that this time corresponds to the end of stage II.

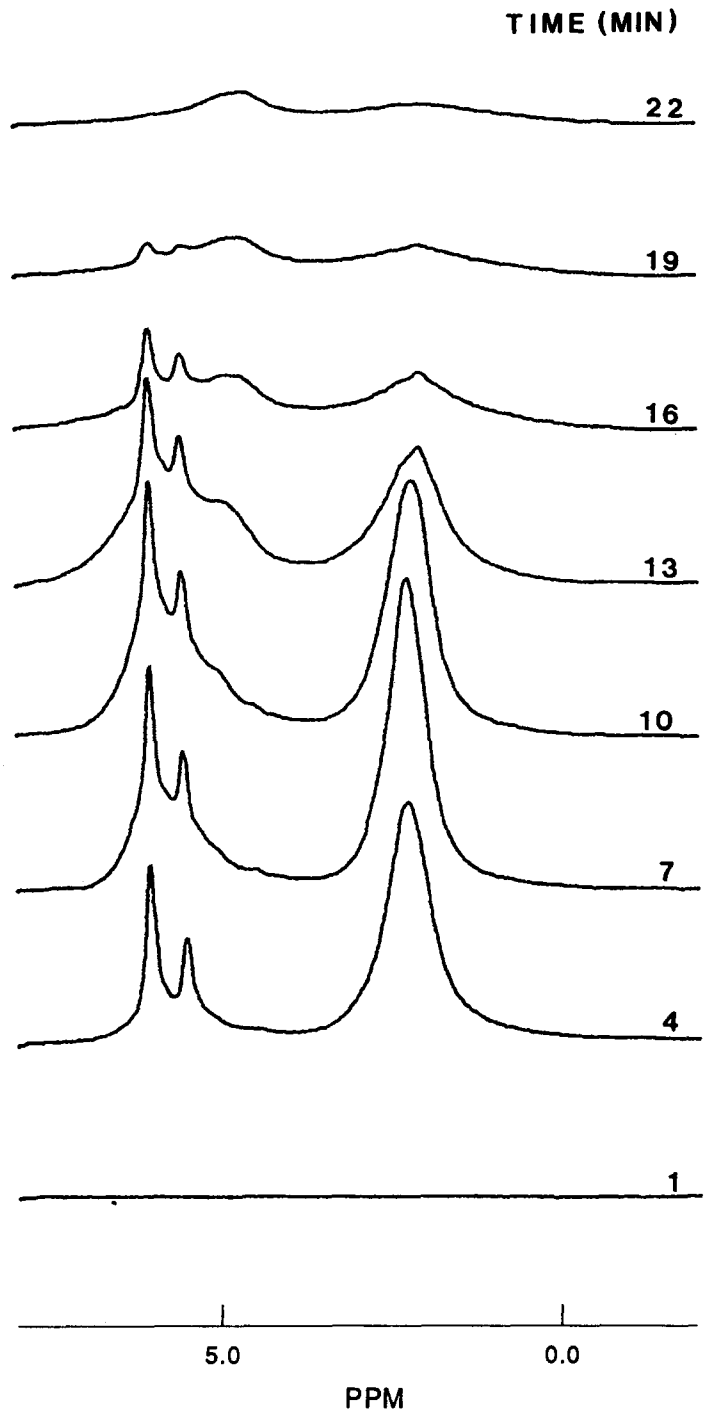

Fig. 4. Time dependence of the ^{1}H-NMR spectra of the D_2O–AA–MBAA–TMED–ASP reaction mixture frozen by method B, $\Delta T = -15$ K.

We believe that the disappearance of the signals due to comonomers in the ^{1}H-NMR spectra (Fig. 4) at stage III and the changes of the above discussed ^{2}H line width both result from the increase of viscosity in the liquid microphase and consequent reduction of molecular mobility when even a small amount of the polymer is formed. In fact, no sharp resonances of AA protons are observed in the spectrum of a frozen model solution consisting of AA (2 wt%) and PAA (1 wt%).

CONCLUSION

CryoPAAG formation in frozen aqueous solutions involves intricate chemical and physical processes. This work clarifies some features of the phenomena.

It is established by ^{1}H- and ^{2}H-NMR that, at $\Delta T = -10 \cdots -25$ K in a frozen aqueous solution of AA–MBAA–TMED, there is a liquid microphase which is formed because of the presence of TMED in the initial solution and contains only 6–3% of the total AA + MBAA content in the starting mixture. Also, in the microphase there is a small amount of unfrozen water (2.6–0.2% of the initial quantity) and the concentration of comonomers there is between 2.5 and 11 times larger than that in the solution before freezing.

At $\Delta T = -15 \cdots -25$ K, the NMR spectra of AA–MBAA–TMED and PAA solutions differ greatly. In the latter case, the amount of unfrozen water is between four and eight times that in the former system, but the D_2O–PAA liquid microphase seems to have greater viscosity resulting in appreciable NMR line broadening. In the NMR spectra of the frozen solution of AA–MBAA–TMED–ASP, an increase in unfrozen water content and in the line width is also found as a result of cryoPAAG formation.

Confirmation of the influence of the method used for freezing the reaction mixture on the initial rate of cryoPAAG formation has also been found. Thus, at $\Delta T = -10 \cdots -20$ K the least duration of the NMR spectra changes, reflecting the initial stage of the polymer formation, is observed after freezing the reaction solution in liquid N_2.

Unfortunately, because of the obvious limitation of the high resolution NMR techniques, it remains unclear in what way the main quantity of comonomers, being in the solid state, becomes involved in cryopolymerization. Assumptions supposing the possibility of the diffusional migration or comonomer molecules through the solid phase to the liquid microphase or the expansion of the reaction "zones" are not completely satisfactory. Solving this problem requires the application of other experimental approaches.

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