Polymer 49 (2008) 3855–3858

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Polymer Communication

In situ ^{1}H NMR studies of free radical cryopolymerization

Harald Kirsebom [a], Gabriel Rata [b], Daniel Topgaard [b], Bo Mattiasson [a], Igor Yu. Galaev [a,*]

[a] Department of Biotechnology, Lund University, SE-221 00 Lund, Sweden
[b] Department of Physical Chemistry 1, Lund University, SE-221 00 Lund, Sweden

ARTICLE INFO

Article history:
Received 13 May 2008
Received in revised form 18 June 2008
Accepted 26 June 2008
Available online 5 July 2008

Keywords:
In situ NMR study
Cryopolymerization
Semi-frozen state

ABSTRACT

Free radical polymerization of DMAAm (dimethylacrylamide)-*co*-PEG diacrylate in a semi-frozen aqueous solution was studied using ^{1}H NMR, which enables to monitor the non-frozen water as well as the reaction of monomers over time. It was possible to confirm the presence of a heterogeneous system with a liquid phase where the monomers were concentrated and the reaction proceeded. The resulting macroporous polymeric structure with dense pore walls was confirmed using scanning electron microscopy (SEM).

1. Introduction

Hydrophilic macroporous materials with interconnected pores have considerable potential for applications as scaffolds for tissue engineering or as continuous phases for bio-chromatography [1–3]. These materials can be prepared using various techniques, such as freeze-drying, salt leaching or cryogelation [4–6]. Cryogelation is based on the formation of a polymeric structure in a semi-frozen state, using ice crystals as a pore-forming agent [7]. On freezing of an aqueous solution of monomers and initiator, the dissolved substances will be enriched in a non-frozen liquid phase surrounded by ice crystals, a process commonly called cryo-concentration [7]. Cryo-concentration is responsible for the polymerization reaction proceeding under highly unusual conditions of low temperature and high monomer concentration, resulting in the unique structure and properties of the gel, a so-called cryogel. When the system is thawed, the melted ice crystals leave behind an interconnected macroporous network with pores in the range of 1–100 μm. Due to the effect of cryo-concentration the pore walls are composed of a dense polymer phase, making the resulting cryogels mechanically strong, elastic and spongy. The unique combination of cryogel properties makes them very attractive as materials for the separation of mammalian cells or bacteria, the isolation of biomolecules, as bioreactors for the cultivation of mammalian cells, or as scaffolds for tissue engineering applications [6,8–10].

As polymerization proceeds in an apparently frozen heterogeneous reaction medium composed of ice crystals and a non-frozen, liquid phase, it is extremely difficult to monitor the reaction and hence elucidate the factors affecting the pore structure. Nuclear magnetic resonance (NMR) may be useful in studying these systems since the method should allow direct investigation of the processes occurring in the liquid phase existing in the apparently frozen sample. This technique may enable the study and quantification of the reacting monomers, as well as studies of the lag phase of the reaction. To the best of our knowledge, only one previous attempt has been made, in 1993 [11], to study cryopolymerization using ^{1}H and ^{2}H NMR. That study was limited to the analysis of the liquid water peak only, as the resolution was not sufficient to enable quantification of the monomers or studies of the polymer. Moreover, some of the samples had first been frozen in liquid nitrogen, immediately converting the water and dissolved substances into a glassy state [12–14], followed by spontaneous crystallization upon the increase in temperature to the range of temperatures studied i.e. −25 to −10 °C. The structure of ice crystals formed from glassy water upon increasing the temperature and that of ice formed during relatively slow freezing of an aqueous solution are fundamentally different [12], despite the fact that the NMR studies are carried out at the same temperature.

In the present study, ^{1}H NMR was used to monitor the amount of non-frozen phase and the progress of the polymerization reaction in situ in samples frozen at −12 °C directly in the spectrometer. The polymerization system studied was the cross-linking polymerization of dimethylacrylamide (DMAAm) using PEG diacrylate as the cross-linker.

* Corresponding author. Department of Biotechnology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden. Tel.: +46 46 2220881; fax: +46 46 2224713.
E-mail address: Igor.galaev@biotek.lu.se (I.Yu. Galaev).

0032-3861/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2008.06.053

2. Experimental

2.1. Materials

N,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED), ammonium persulfate (APS), DMAAm, PEG diacrylate (average molecular weight 258 g mol^{-1}), silver iodide were all obtained from Aldrich. MilliQ purified water was used for the preparation of all solutions.

2.2. Measurement of cryopolymerization

The monomers (6% w/v) with DMAAm/PEG diacrylate at molar ratios of 5:1 or 60:1, representing highly and weakly cross-linked samples, respectively, were dissolved in water. An activator TEMED, 2% (w/v) of the total monomer weight was added and the solution was degassed under vacuum to remove any dissolved oxygen which inhibits the radical reaction, after which the solution was stored on ice and an initiator APS, 2% (w/v) of the total monomer weight was added in order to start the reaction. The reaction solution (400 μl) was immediately transferred to a NMR tube (diameter 5 mm) containing a few crystals of silver iodide, which were added to promote ice crystal nucleation. Silver iodide does not interfere with either the reaction or with the NMR measurements as the crystals were located well below the active volume of the radiofrequency coil.

The reaction was immediately monitored under controlled temperature at −12 °C. The measurements were carried out with a Bruker DSX 200 MHz spectrometer. The sequence used in investigations was a 90° pulse sequence, with a recycle delay of 1 s, 4 scans, and a pulse length of 12 μs. The experimental time was 5 s, and the ^{1}H spectra were acquired every 20 s for the first 30 min and thereafter spectra were acquired with 10 min delay for a total time of about 14 h.

2.3. Scanning electron microscopy

Samples prepared in NMR tubes were cut into discs for SEM and were fixed in 2.5% glutaraldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4) over night at 4 °C. The samples were dehydrated in ethanol (0, 20, 75, 95 and 99.5%) and then critical-point dried. The dried samples were sputter-coated with gold/palladium (40/60) and examined using a JEOL JSM-5000LV scanning microscopy.

3. Results and discussion

Freezing of the polymerization system results in the formation of a network of ice crystals surrounded by a small amount of non-frozen liquid with accumulated solutes. The amount of non-frozen water in semi-frozen samples was quantified as a function of time by measuring the area of the water peak during the reaction, while the polymerization of the monomers was monitored by measuring the decrease in the area of a peak representing the vinyl group of the monomers (Fig. 1).

The areas of the peaks were quantified by fitting a number of Lorentzian functions to the spectra. The peaks at 6.9, 6.3 and 6.0 ppm correspond to the protons in the vinyl group of the monomer. The peak at 6.9 ppm is most suitable for monitoring the polymerization reaction as it is well separated best from the water peak and could be better fitted by the Lorentzian function.

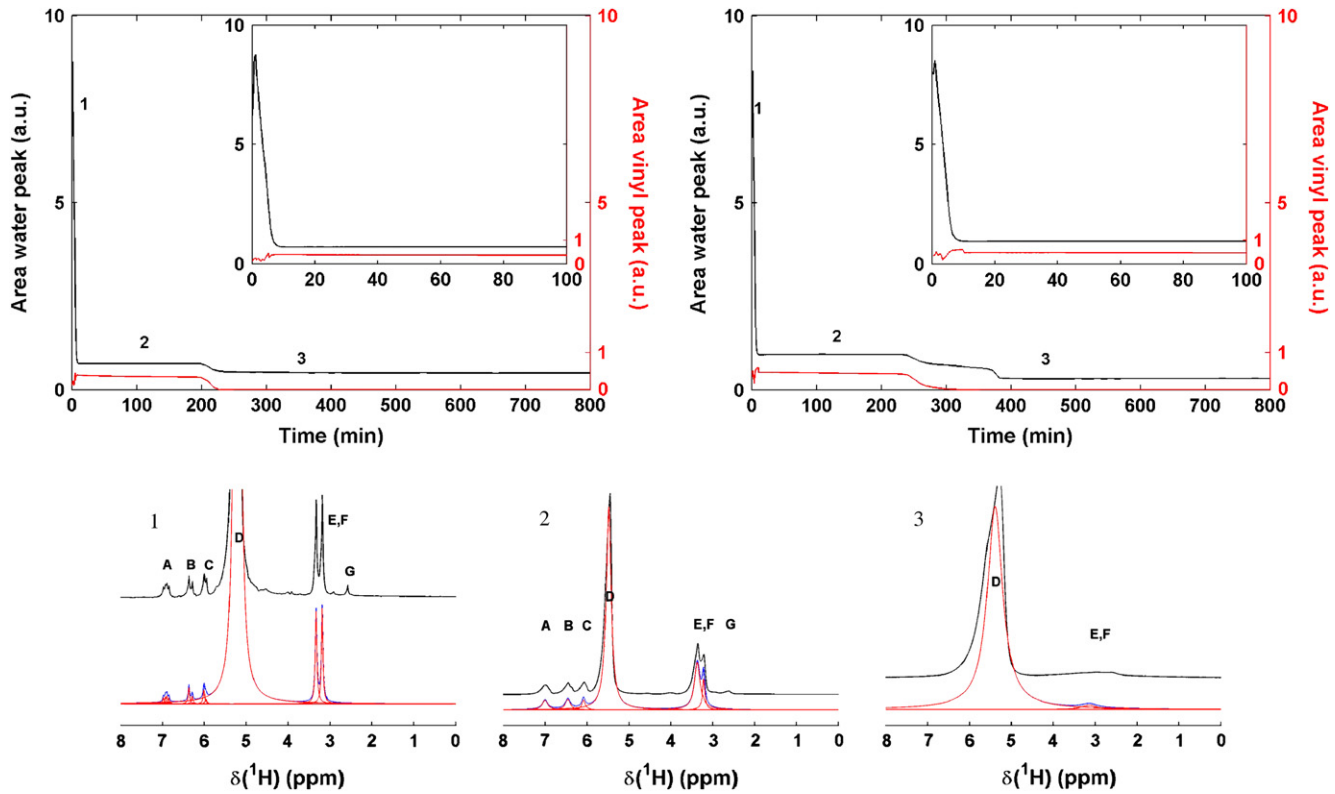

Fig. 1. Results of measurements on samples with different cross-linking degree (6% DMAAm/PEG diacrylate) (5:1) top left and (60:1) top right studied at −12 ± 1 °C in a Bruker DMX 200 spectrometer operating at a ^{1}H frequency of 200.13 MHz. ^{1}H spectra were acquired every 25 s for the first 30 min, and thereafter at 10-min intervals. Top figures shows the change in area of the water peak (black) and the vinyl peak at 6.9 ppm (red) during the reaction. (1) Shows the results obtained before freezing the sample, (2) shows the results from the frozen sample and (3) the spectra after completion of the polymerization reaction. A, B and C denote the protons in the vinyl group of the monomer, D the water peak, E and F the methyl peaks an G the signal from the activator TEMED. The black lines in (1)–(3) show the recorded spectra, the red lines show the deconvoluted spectra with Lorentzian functions fitted to each peak, and the blue lines show the sum of the deconvoluted spectra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

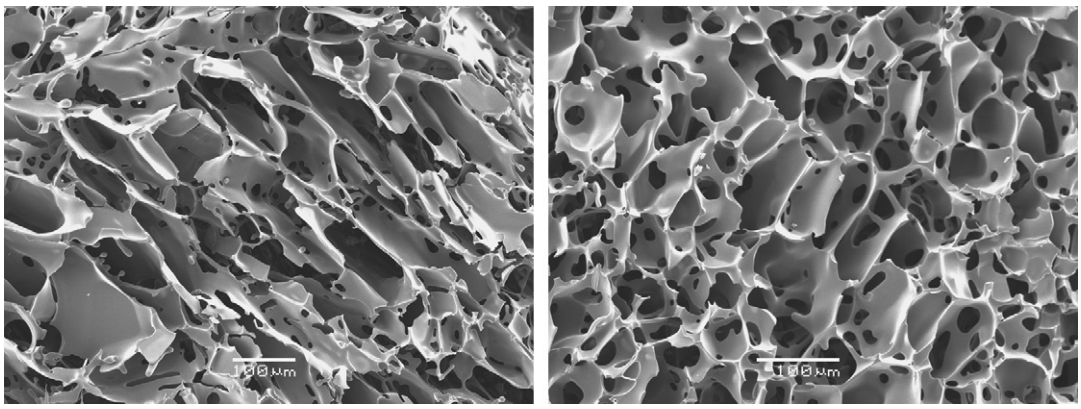

Fig. 2. Scanning electron micrographs of 6% DMAAm/PEG diacrylate cryogels prepared at −12 °C in situ in the NMR spectrometer. Left: micrograph of a highly cross-linked cryogel (5:1 mol/mol), right: weakly cross-linked cryogel (60:1 mol/mol). Samples were fixed in 2.5% glutaraldehyde and gradually dehydrated with EtOH before critical-point drying in order minimize changes in the structure.

Peaks from the vinyl groups of cross-linker are only clearly visible in highly cross-linked samples as they partly overlap the DMAAm peaks. However, two separated peaks of PEG diacrylate are clearly visible at 3.9 and 4.43 ppm. In all cases, the samples were frozen within a few minutes, which can be seen as the sharp decrease in the water signal in Fig. 1. The presence of a liquid phase in the apparently frozen polymerization feed at −12 °C is demonstrated by a clearly visible water signal, which was not observed when pure water was frozen at the same temperature. The ^{1}H spectra showed complete conversion of the vinyl peaks in the system despite the fact that the average gel yields of the reactions were approximately 85%, indicating that part of the polymer formed was not incorporated into the gel structure but was washed out of the macroporous structure. Spectra of both the highly and weakly cross-linked samples after polymerization showed a characteristic broad peak in the region of the methyl group, which was more pronounced for the weakly cross-linked sample indicating that this is a more mobile and flexible system.

The signals from the monomers broadened in the semi-frozen state as a result of the increasing rotational correlation time upon decreasing temperature and differences in magnetic susceptibility between the coexisting fluid and solid phases. However, the areas of the peaks appeared to be unchanged upon initial freezing, indicating that most of the monomers were concentrated in the liquid phase rather than embedded in ice. Approximately 8–9% of the water remained unfrozen at −12 °C in both systems prior to the onset of polymerization. The amount of unfrozen water is defined by the initial molality of the reaction medium. Using an ideal approximation [15]:

$$\Delta T = K_f b$$

where ΔT is the depression in freezing point, K_f is the cryoscopic constant for water (1.86 K kg mol^{-1}), and b the molality of the solutes, it is possible to calculate the freezing point depression of the cryoconcentrated liquid phase in the polymerization feed. The calculated molalities of sample 5:1 and 60:1 in the non-frozen phase were 5.9 and 6 mol/kg. From the molality the freezing point depression can be obtained, 11 K, agrees very well with the freezing temperature used, namely −12 °C. As this monomer solution in water is not an ideal solution, the freezing point depression will be slightly greater [15]. Thus, the amount of non-frozen liquid phase is determined only by the depression in freezing point.

As the polymerization reaction proceeds, the molality of the liquid phase decreases due to the conversion of individual monomer molecules into the cross-linked polymer, and the effect of freezing point depression becomes negligible, according to the equation above. However, approximately 5% of the initial water remained unfrozen even after completion of the polymerization reaction. This amount of non-frozen water corresponds to approximately 5 water molecules per monomer unit of polymer, and the non-frozen water is most probably associated with the polymer.

A lag phase of between 3 and 4 h was observed before the reaction took place in all the systems studied. This is significantly longer than that observed for the same reactions at room temperature, probably due to the slow formation of free radicals at −12 °C. The onset of polymerization is clearly visible as the intensity of the signals from the monomers and the amount of non-frozen water decrease. We currently have no explanation of the rapid decrease in the amount of non-frozen water during the final stages of polymerization.

The formation of a macroporous structure was confirmed in both highly and weakly cross-linked gels using scanning electron microscopy (Fig. 2).

The pore walls show a smooth texture without any apparent microporosity. The thickness of the pore walls and size of the pores in the two samples seemed to be rather similar, and thus cross-linking density appeared not to affect the structure as revealed by SEM. However a higher mechanical stability was obtained for the 5:1 sample compared to the 60:1; this might be due to more cross-linked sample.

4. Conclusions

These studies have shown that ^{1}HNMR is a highly promising technique for studying the formation of cryogels in situ as both the changes in the amount of non-frozen water and the progress of the polymerization reaction can be monitored. The behavior of water is of crucial importance for the formation of well structured cryogels. NMR is an ideal technique to study the behavior of water and the solutes in the non-frozen water phase. Studies are underway to determine the applicability of NMR to monitor the cryopolymerization of different monomers, as well as cryogel formation via cross-linking of pre-formed polymers.

Acknowledgment

Part of this work was financially supported by the Crafoord Foundation, Sweden, Swedish Research Council, Project 70362401 and by the FP-6 Project, Biomaterials for Wound Healing and Tissue Regeneration, MKTI-CT-2006-042768.

References

[1] Jungbauer A, Hahn R. J Sep Sci 2004;27:767–78.
[2] Hutmacher DW. Biomaterials 2000;21:2529–43.
[3] Lee KY, Mooney DJ. Chem Rev 2001;101:1869–79.
[4] Wu L, Ding J. Biomaterials 2004;25:5821–30.
[5] Huang Y, Onyeri S, Siewe M, Moshfeghian A, Madihally SV. Biomaterials 2005; 26:7616–27.
[6] Dainiak MB, Kumar A, Galaev IY, Mattiasson B. Proc Natl Acad Sci U S A 2006; 103:849–54.
[7] Lozinsky VI, Galaev IY, Plieva FM, Savina IN, Jungvid H, Mattiasson B. Trends Biotechnol 2003;21:445–51.
[8] Galaev IY, Dainiak MB, Plieva F, Mattiasson B. Langmuir 2007;23: 35–40.
[9] Bansal V, Roychoudhury PK, Mattiasson B, Kumar A. J Mol Recognit 2006;19: 332–9.
[10] Bölgen N, Plieva F, Galaev IY, Mattiasson B, Piskin E. J Biomater Sci Polym Ed 2007;18:1165–79.
[11] Gusev DG, Lozinsky VI, Bakhmutov VI. Eur Polym J 1993;29:49–55.
[12] Debenedetti PG, Stanley HE. Phys Today 2003:40–6.
[13] Ludwig R. Angew Chem Int Ed 2006;45:3402–5.
[14] Mishima O, Stanley HE. Nature 1998;396:329–35.
[15] Fullerton GD, Keener CR, Cameron IL. J Biochem Biophys Methods 1994;29: 217–35.