Iranian Polymer Journal
16 (1), 2007, 13-20

Available online at: http://journal.ippi.ac.ir

Monitoring of Polyethylene Glycol-diacrylate-based Hydrogel Formation by Real Time NMR Spectroscopy

Mohammad Imani*[1], Shahriar Sharifi[2], Hamid Mirzadeh[1,2], and Farshid Ziaee[1]

(1) Department of Novel Drug Delivery Systems, Iran Polymer and Petrochemical Institute, P.O. Box: 14965/115, Tehran, Iran
(2) Biomedical Engineering Department, Amirkabir University of Technology, P.O. Box: 15875/4413, Tehran, Iran

Received 8 April 2006; accepted 23 January 2007

ABSTRACT

Hydrogels produced by in situ polymerization have been extensively studied as biomaterials in applications such as scaffolds for tissue engineering and drug delivery systems. In situ polymerization process allows the hydrogel to be generated in vitro or in vivo from a low viscosity solution of monomer or low molecular weight polymer by free radical pathway. In this study, polyethylene glycol-diacrylate (PEG-DA) macromer has been synthesized by esterification of PEG with acryloyl chloride and characterized by NMR and FTIR spectroscopies and swelling study. Cross-linking was initiated by a pair of redox initiator, i.e. ammonium persulphate and *N,N,N′,N′*-tetramethylethylenediamine. The conversion rate of the macromers to a cross-linked network was determined using ^{1}H NMR and dynamic oscillatory test at 37ºC and continuous disappearance of vinyl group upon addition of redox agent to a macromer solution was evaluated.

Key Words:

hydrogels;
in situ polymerization;
kinetics;
PEG-diacrylate;
real-time NMR.

INTRODUCTION

Hydrogels have been extensively investigated in pharmacy and medicine due to their high water absorption, low interfacial tension that they demonstrate when surrounded by biological tissues and ability to respond to specific environmental stimuli such as pH, ionic strength and temperature [1,2]. They are also promising because of the similarities between their physical properties and those of living tissues [3-5].

In situ polymerization process to produce hydrogels with biological applications can be regarded as

(*) To whom correspondence to be addressed.
E-mail: m.imani@ippi.ac.ir

in vitro or in vivo formation of higher molecular weight products from syringeable low viscosity solutions of monomers, oligomers or macromers in a minimally invasive manner. Cross-linked hydrogel networks can be formed in situ in a variety of ways including free radical reactions initiated by heat, absorption of photons or ionic interactions between small cations and anions [6,7]. The cross-linking can result still in hydrogels with high water content possessing mechanical properties similar to those of soft tissues [8]. There are some challenges accompanied with this approach such as burst release of loaded agent; which have limited its clinical application. The burst is due to the lag time for solidification of the polymer, i.e. during the progress of the reaction when the polymer is still liquid-like which facilitates the drug diffusion [9-11]. Cross-linking time should be reduced in order to reduce the burst effect.

Redox initiation systems can be used as an effective way of generating free radicals under mild and aqueous conditions [12,13]. The water-soluble initiator e.g., ammonium persulphate (APS) when mixed with a reducer yields a sulphate anion plus a radical ion which can cross-link the macromers in less than 10 min at 37ºC [14]. The rapid release of the loaded agent will be reduced due to a reduction in the reaction time and will follow diffusion-controlled release kinetics through polymeric matrix in most part of the device life cycle. NMR Spectroscopy has been used as an effective method to investigate cross-linking, cross-link density and the kinetics [15-18]. Real time observation of the reaction kinetics by NMR spectroscopy can be promising if there was a characteristic bond either of monomer or polymer which changes in intensity by the progress of the polymerization. For the vinyl containing macromer the C=C bond exhibits such behaviour that can be used for this purpose.

In this research, in order to evaluate the kinetics profile of polyethylene glycol diacrylate (PEGDA) hydrogel formation; the real time ^{1}H NMR spectroscopy has been used to probe the solution polymerization of macromers in the presence of APS and activator in deuterated water. The results were compared qualitatively by rheological studies. The macromers and the resulted hydrogel networks were also characterized by FTIR and ^{1}H NMR spectroscopies and swelling studies, respectively.

EXPERIMENTAL

Materials

Polyethylene glycol with molecular weight of 600 (PEG), acryloyl chloride, toluene, methylene chloride and D_2O (99% deuterated) all were supplied by Merck (Darmstadt, Germany). Triethylamine (TEA), ammonium persulphate (APS) and *N,N,N',N'*-tetramethylethylenediamine (TMEDA) were purchased from Aldrich (Milwaukee, USA) and used without further purification. Deionized water was prepared freshly by reverse osmosis. The other reagents were all supplied by Merck and used as received.

Macromer Synthesis

PEG was purified prior to synthesis by azeotropic distillation in toluene. 0.2 Mol of PEG, 0.4 mol of TEA and 300 mL of methylene chloride were charged into a 500 mL four-necked flask equipped with a mechanical stirrer. Acryloyl chloride (0.4 mol) was added dropwise; while the temperature was maintained at 0ºC. Esterification proceeded at room temperature for 6 h. The precipitated triethylamine hydrochloride salt was removed by extraction with distilled water. The product then washed with 0.1 M NaOH solution and deionized water for several times until it became completely neutral. Residual water was removed from the organic phase by treatment with anhydrous magnesium sulphate. A clear liquid of PEGDA with a yield of 80% was obtained by rotary evaporation.

Characterization of Macromers

High resolution 400 MHz ^{1}H NMR spectra were recorded in deuterated chloroform on a 400 MHz Bruker spectrometer. All runs were performed at room temperature, 20 Hz sample spinning, 90 tip angle for the observation pulse and 6 s pulse delay for each scan. FTIR Spectroscopy (Bruker, Equinox 55) was also used in order to evaluate the acylation reaction and presence of vinyl double bonds in structure.

Preparation of Hydrogels

The hydrogels were prepared from the synthesized PEGDA macromer with molecular weight of 720 g.mol^{-1} which was determined by ^{1}H NMR. The redox initiation system for the cross-linking reaction consisted of APS combined with an accelerator,

TMEDA. Typically, 0.5 g of macromer was dissolved completely in a 2 mL of accelerator solution (25 mM) and poured into plastic mould. Then 2 mL of APS aqueous solution (25 mM) was added to this mixture using disposable syringe. The reaction was conducted at 37ºC in water bath. After 2 h the hydrogels were removed from the mould.

To measure the hydrogels sol fraction; the specimens were placed in an oven at 40ºC overnight to dry completely. The dried samples were accurately weighed (W_i) and then placed into a 200 mL of deionized water to extract the water soluble unreacted fraction. After 48 h, the specimens were removed from water and dried to constant weight (W_d) at 40ºC. The sol fraction was calculated using following formula:

$$\text{Sol fraction} = \frac{W_i - W_d}{W_i} \quad (1)$$

The sol fraction of samples was averagely 4.7%± 1.7.

Dynamic Swelling Studies

Dynamic swelling studies were performed on hydrogels to evaluate the time dependence behaviour of swelling with pH. The samples were prepared according to the above mentioned method in teflon moulds immersed at 37ºC water bath and then dried in oven for 8 h. The final samples were disks of 10 mm diameter and 3 mm height.

Disks were weighed and placed in a phosphate buffer solution with pH values of 3 and 7 at room temperature and then taken out of the solution at time intervals, blotted for removal of the surface water and weighed. The swelling of the network can be expressed by the weight swelling ratio, Q:

$$Q = W_s/W_d \quad (2)$$

where W_s is the weight of the swollen hydrogel and W_d is the weight of the initially dried hydrogel.

Rheology Experiments

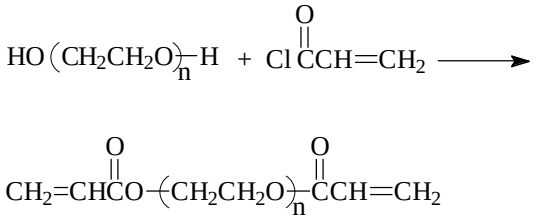
In situ rheology experiments were used to assess the reaction kinetics. Modulus of the complex viscosity (η^*) of these solutions were monitored via a dynamic oscillatory test at 37°C with a rheometer (Physica MCR 300). Typically, the polymer solution was placed in a stainless steel mould (9 mm in diameter and 12 mm in depth) so that it filled 10 mm of the mould depth. The parallel plate (8 mm diameter) was lowered into the mould until it contacted the surface of the solution. Gelation onset was determined when G" (loss modulus) crossed over G' (storage modulus).

NMR Studies

^{1}H NMR Spectra (1 scan with 6 s delay) were recorded at 400 MHz and 37ºC. The observed changes in the intensity of the vinyl protons were determined as a function of time. One millilitre of 25 mM TMEDA solution in deuterated water was prepared and 50 µL of the macromers was dissolved in that and poured into 5 mm diameter NMR tube. One millilitre of 25 mM solution of APS in deuterated water was then added to the tube and immediately the tube was transferred to NMR spectrophotometer. The spectrum was recorded as a function of time.

RESULTS AND DISCUSSION

PEGDA is prepared by the acylation of PEG and acryloyl chloride, as shown below.



FTIR Spectrum of PEGDA is shown in Figure 1. Absorption of the C=C bonds occurs at 1633 cm^{-1} and the carbonyl groups at 1724 cm^{-1}. ^{1}H NMR Spectrum of the macromer is shown in Figure 2 and the peak assignments are shown on the spectrum. In order to show that all of the terminal hydroxyl functional groups of PEG reacted with acryloyl chloride and there is no remaining free hydroxyl group we used trifluoroacetic anhydride; a compound that immediately reacts with free hydroxyl groups to form trifluoroacetate esters [19]. By formation of trifluoroacetate, the hydroxyl signal peak should be disappeared. Since there was no special change in ^{1}H NMR spectra after adding trifluoroacetic in comparison to the untreated state, it can be concluded that there is no free hydroxyl groups. Two additional peaks at 1 δ and 2.5 δ are

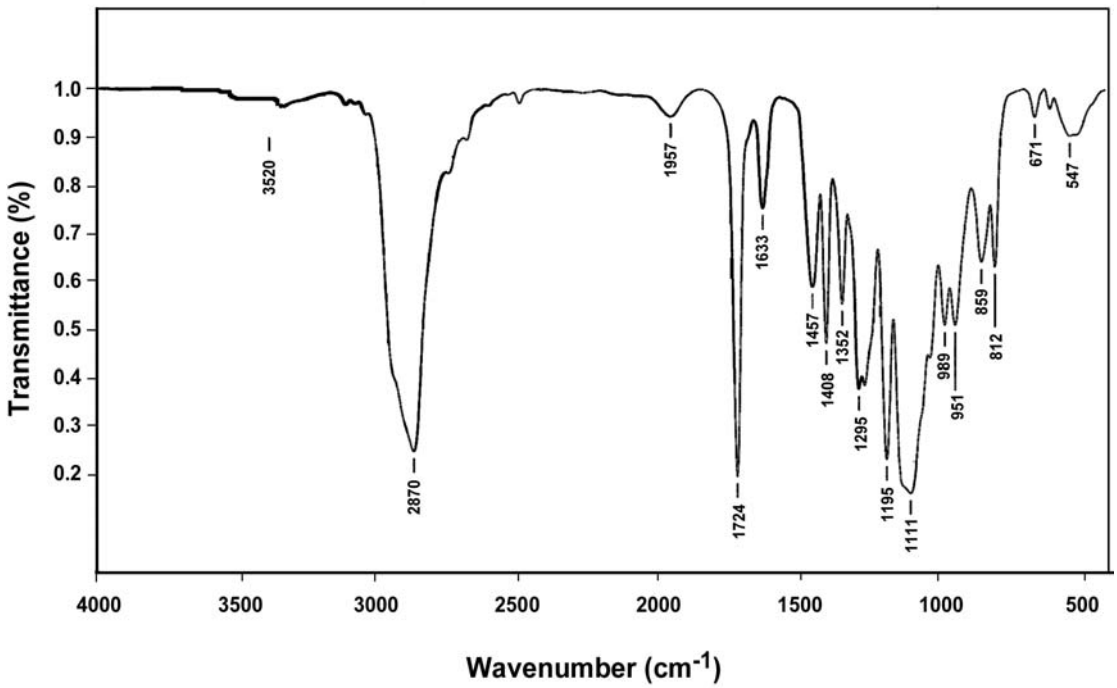


Figure 1. FTIR Spectrum of PEGDA.

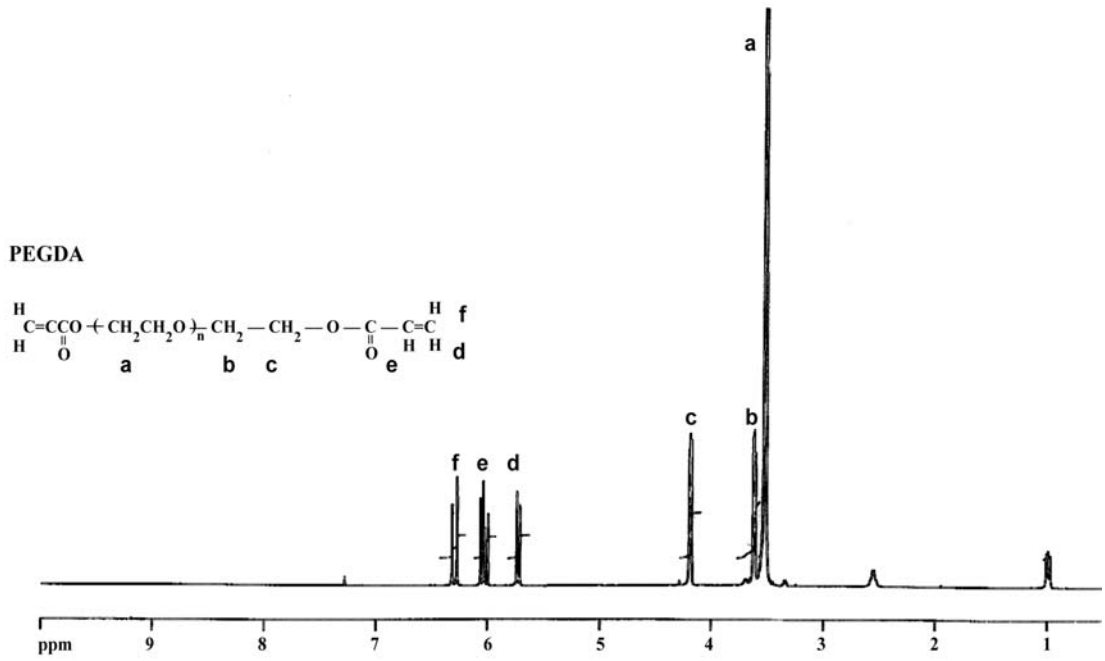
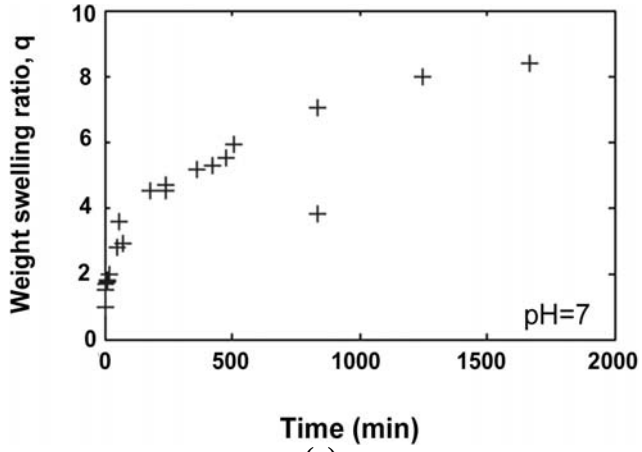


Figure 2. H NMR Spectrum of PEGDA.

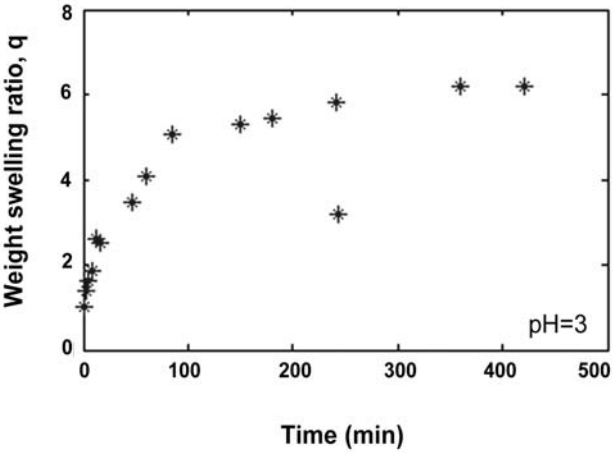
assigned to the remaining TEA which has been used as a catalyst to neutralize librated HCl in the acylation process.

Dynamic swelling studies of the prepared hydrogels in buffers of pH values of 3 and 7 have been depicted in Figure 3.

It was observed that PEGDA based hydrogels exhibit low pH-responsive swelling behaviour. In this case, according to NMR and FTIR spectra (Figures 1 and 2) there is no ionizable functional group in the macromers and consequently in resulting hydrogel, there will be no significant differences between

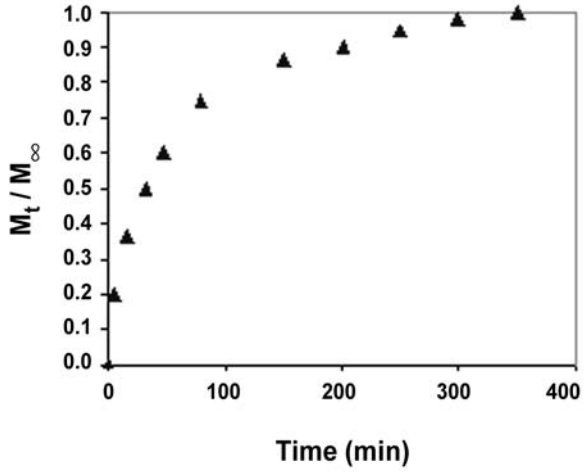


(a)

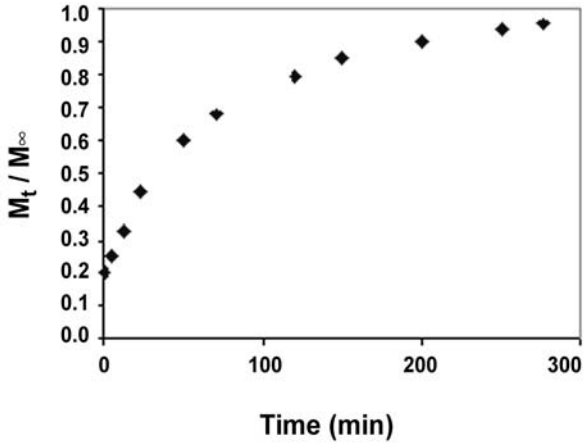


(b)

Figure 3. Swelling behaviour of PEGDA based hydrogels in: (a) pH=7; and (b) pH=3.



(a)



(b)

Figure 4. Fractional water uptake of hydrogels in different pHs: (a) pH=7; and (b) pH=3.

swelling behaviours in pH values of 7 and 3. According to Flory-Rehner theory the swelling of this hydrogel is due to a balance between opposite forces, i.e. thermodynamic force of mixing and the retractive force of polymer chains (Figure 3).

In Figure 4, M_t/M_∞, i.e. fractional water uptake versus time has been plotted in different pH values where M_t is the mass of water absorbed at time t and M_∞ is at equilibrium.

In order to investigate the mode of penetrant transport mechanism through hydrogel; the portion of the curve with $M_t/M_\infty < 0.6$ was analyzed using the following equation:

$$M_t/M_\infty = kt^n \quad (3)$$

where k is a characteristics constant of the hydrogel and n is an exponent describing the mode of penetrant transport mechanism which for cylindrical geometries will be 0.45 in case I (Fickian), 0.45 to 0.89 in non-Fickian (anomalous) and will be equal to 0.89 in relaxation transport-controlled mechanisms [20]. The constants n and k were calculated from the slopes and intercepts of $Ln(M_t/M_\infty)$ versus Lnt plots from the experimental data shown in Figure 4 and calculated values of n and k are shown in Table 1.

Table 1. Parameters of n and k for PEGDA hydrogels swollen in buffer solution with different pHs at 25ºC.

n		k	
pH = 3	pH = 7	pH = 3	pH = 7
0.48	0.52	0.05	0.04

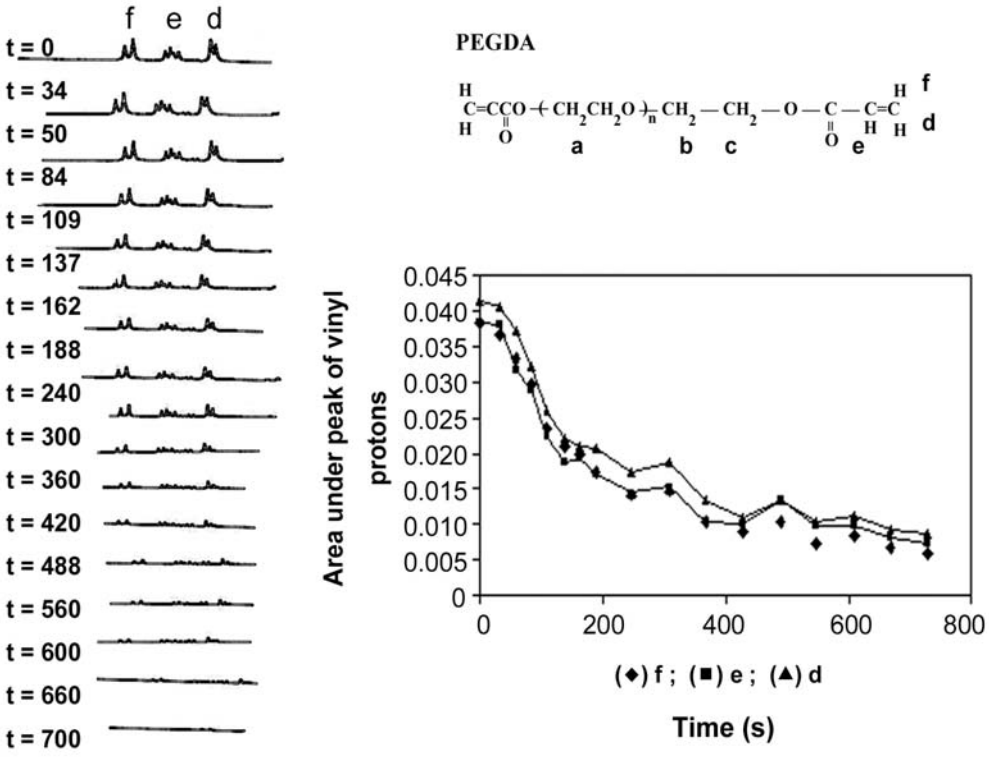


Figure 5. Real time ^{1}H NMR evaluation of the reaction progress: (a) observed ^{1}H NMR peaks for terminal vinyl protons, and (b) changes in peaks integral during reaction time course. The structure is shown in (c).

The value of n at pH 7 (i.e., 0.52) indicates that the transport mechanism is almost anomalous or non-Fickian and at pH 3 will be equal to 0.48 which means that it obeys nearly the same mechanism and both are in agreement with dynamic swelling studies previously discussed (Figure 3).

A real time NMR spectroscopy method was developed in order to evaluate the kinetics of network formation. Remarkable changes in the peak intensities of terminal vinyl protons in comparison to PEG related CH_2CH_2O peak on the polymer backbone were observed during the reaction time period which can be correlated to the conversion (Figures 5 and 6). The following equation was used to calculate the conversion rate of PEGDA to cross-linked hydrogel.

where VPI is the terminal vinyl proton integral and

$$\text{Conversion}\,(\%) = \frac{\text{VPI}_{T=0} - \text{VPI}_{T=t}}{\text{VPI}_{T=0}} \times 100 \qquad (4)$$

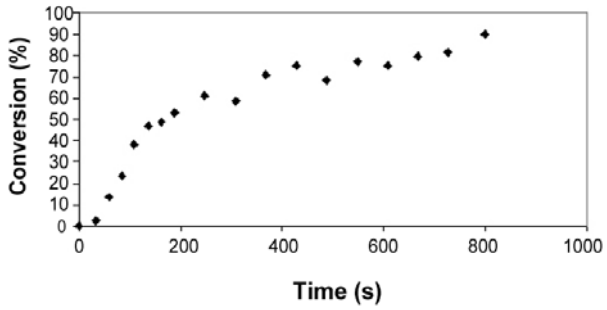


Figure 6. Conversion (%) versus time calculated from ^{1}H NMR peak integrals.

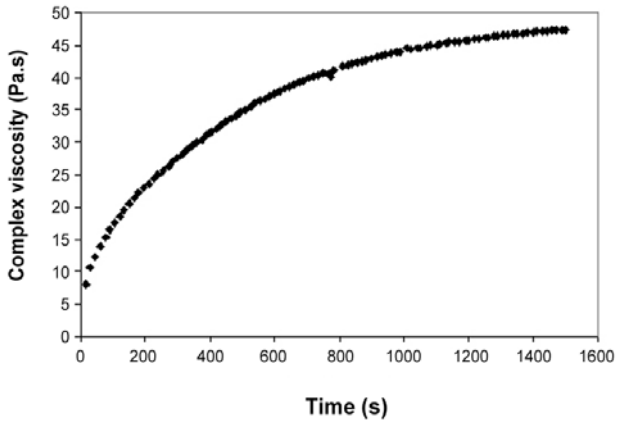


Figure 7. Changes in complex viscosity during the PEGDA cross-linking reaction.

T is the time course from zero to t. The kinetics profile shows that the reaction goes up to 90% completion in approximately 16 min (Figure 6).

The reaction takes place in two successive phases; the initiation and propagation steps observed in the first 5 min are known to occur due to formation of free radicals. The second phase can be distinguished after 5 min where the slope of conversion versus time curve is decreased probably due to increased viscosity and restricted motions of polymeric radicals at higher conversions. These results are in agreement with complex viscosity followed up the same reaction (Figure 7) which shows the profile of increasing viscosity during time in two distinct phases. For PEGDA formulations (Figure 7) gelation onset occurs within 4 min at 37°C.

CONCLUSION

PEGDA is a liquid macromer with the ability of in situ cross-linking which can be synthesized by acylation of PEG with acryloyl chloride in the presence of TEA. In order to reduce the burst of an in situ forming controlled release device, the system should have the low setting time. Rheological and NMR spectroscopic studies showed that PEGDA could be cross-linked with the redox initiation system within approximately 5 min which can be regarded a suitable time.

REFERENCES

1. Park K., Shalaby W., Park H., *Biodegradable Hydrogels for Drug Delivery*, CRC, 1993.
2. Ottenbrite R.M., Huang S.J., Park K., Hydrogels and biodegradable polymers for bioapplications, *ACS Symp. Ser. No. 627*, 1996.
3. Peppas N.A., *Hydrogels in Medicine and Pharmacy*, CRC, Boca Raton, FL, USA, 1987.
4. Dusek K., Prins W., Structure and elasticity of non-crystalline polymer networks, *Adv. Polym. Sci.*, **6**, 1-102, 1969.
5. Russo P.S., A prospective on reversible gels and related systems, in reversible polymeric gels and related systems, *ACS Symp. Ser.*, **350**, 1-21, 1987.
6. Hatefi A., Amsden B., Biodegradable injectable in situ forming drug delivery systems, *J. Control. Release*, **80**, 9-28, 2002.
7. Temenoff J.S., Mikos A.G., Injectable biodegradable materials for orthopedic tissue engineering, *Biomaterials*, **21**, 2405-2412, 2000.
8. Lin-Gibson S., Bencherif S., Cooper J., Wetzel S.J., Antonucci J.M., Vogel B., Horkay F., Washburn N.R., Synthesis and characterization of PEG dimethacrylates and their hydrogels, *Biomacromolecules*, **5**, 1280-1287, 2004.
9. Moore L.A., Norton R.L., Whitman S.L., Dunn R.L., An injectable biodegradable drug delivery system based on acrylic terminated poly(ε-caprolactone), Presented at the 21st Annual Meeting of the Society of Biomaterials, CA, 1995.
10. Kissel T., Li Y., Unger F., ABA-Triblock copolymers from biodegradable polyester A-blocks and hydrophilic poly(ethylene oxide) B-blocks as a candidate for in situ forming hydrogel delivery systems for proteins, *Adv. Drug Deliv. Rev.*, **54**, 99-134, 2002.
11. Anseth K.S., Metters A.T., Bryant S.J., Martens P.J., Elisseeff J.H., Bowman C.N., In situ forming degradable networks and their application in tissue engineering and drug delivery, *J. Control. Release*, **78**, 199-209, 2002.
12. Sarac A.S., Redox polymerization, *Prog. Polym. Sci.*, **24**, 1149-1204,1999.
13. Allcock H.R., Lampe F.W., *Contemporary Polymer Chemistry*, Prentice Hall, Englewood Cliffs, Nj, 1981.
14. Temenoff J.S., Park H., Jabbari E., Conway D.E., Sheffield T.L., Ambrose C.G., Mikos A.G., Thermally cross-linked oligo(poly(ethylene glycol) fumarate) hydrogels support osteogenic differentiation of encapsulated marrow stromal cells in vitro, *Biomacromolecules*, **5**, 5-10, 2004.
15. Packman L.C., Perham R.N., Roberts G.C.K., Cross-linking and ^{1}H NMR spectroscopy of the pyruvate dehydrogenase complex of Escherichia coli, *Biochem. J.*, **205**, 389-96, 1982.
16. Nieto J.L., Baselga J., Hernandez-Fuentes I., Llorente M.A., Piérola I.F., Polyacrylamide networks. Kinetic and structural studies by high field ^{1}H NMR with polymerization in situ, *Eur. Polym. J.*, **23**, 551-555, 1987.
17. Haw F., Johnson N.A., In-situ curing study of an epoxy resin using nuclear magnetic resonance

with magic-angle spinning, *Anal. Chem.*, **58**, 3254-3256, 1986.
18. Bergmann K., Demmler K., Untersuchung des härtungsablaufs ungesättigter polyesterharze mit hilfe von NMR-messungen, *Colloid Polym. Sci.*, **252**, 193-206, 1974.
19. Gallardo A., San Roman J., Dijkstra P.J., Feijen J., Random polyester transesterification: Prediction of molecular weight and M_w distribution, *Macromolecules*, **31**, 7187-7194, 1998.
20. Kim B., Flamme K.La., Peppas N.A., Dynamic swelling behavior of pH-sensitive anionic hydrogels used for protein delivery, *J. Appl. Polym. Sci.*, **89**, 1606-1613, 2003.