J. Biomater. Sci. Polymer Edn, Vol. 14, No. 3, pp. 227–239 (2003)

Also available online - www.vsppub.com

Crosslinkable PEO-PPO-PEO-based reverse thermo-responsive gels as potentially injectable materials

ALEJANDRO SOSNIK [1,*], DANIEL COHN [1], JULIO SAN ROMÁN [2] and GUSTAVO A. ABRAHAM [2]

[1] *The Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel*

[2] *Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain*

Received 26 August 2002; accepted 19 November 2002

Abstract—This paper describes the functionalization and crosslinking of Pluronic^{RTM} derivatives in aqueous solution at 37° C. Pluronic dimethacrylate was obtained by reacting native PEO-PPO-PEO triblocks with methacryloyl chloride and then crosslinking them by free radical polymerization at 37° C, using a redox system. The resulting gel and its rheological behavior were characterized by different techniques. The swelling study of the crosslinked polymer was indicative of its reverse thermo-responsive behavior, as illustrated by the almost 800% water uptake of the polymer at 37° C, as opposed to the 1600% attained by the polymer at 25° C. As expected, while the Pluronic dimethacrylate gel displayed an E_c value of 142.5 ± 29.7 kPa at 37° C, the crosslinked system attained a Young's modulus three times higher: 415.2 ± 45.7 kPa. Finally, the environmental SEM analysis revealed the porous microstructure of the crosslinked gels.

Key words: Injectable polymers; reverse thermal gelation; PEO-PPO-PEO triblocks; crosslinking; viscoelastic behavior; gels.

INTRODUCTION

The development of polymers suitable for implantation without requiring a surgical procedure has triggered much attention in recent years. The reverse thermo-responsive phenomenon is usually known as reverse thermal gelation (RTG) and it constitutes one of the most promising strategies for the development of injectable systems. The water solutions of these materials display low viscosity at ambient temperature and exhibit a sharp viscosity increase as the temperature rises within a very narrow temperature interval, producing a gel once they reach body temperature. There are a number of reverse thermo-responsive polymers [1–11].

* To whom correspondence should be addressed. E-mail: alejo@pob.huji.ac.il

Among them, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO), commercially available as Pluronic^{RTM} [9–15], is one of the most important and best investigated families of reverse thermo-responsive biomedical polymers. Unfortunately, the levels of viscosity attained by PEO-PPO-PEO gels at 37° C are not high enough for most clinical applications. They are characterized by very high permeabilities, a property which renders them inappropriate for drug delivery applications because of the excessively fast release from these gels [16–18]. This, in addition to unacceptably short *in vivo* residence times, has made them unsuitable for most clinical applications. Consequently, a new generation of reverse thermo-responsive polymers displaying improved properties is called for [19, 20].

The objective of this study was to address the limitations described above, by developing reverse thermo-responsive syringeable systems that would rapidly crosslink *in vivo*, under clinically acceptable conditions. Thus, PEO-PPO-PEO triblocks were functionalized, so that their aqueous solutions combined the following: (i) straightforward injectability due to their low viscosity at ambient temperature; (ii) a sharp increase in viscosity when heated up to 37° C, upon injection into the body; and (iii) a fast *in situ* crosslinking reaction due to the presence of the two reactive groups incorporated into the RTG-displaying polymer. In this context, the work conducted by Pathak *et al.* [21], which photopolymerized acrylate-capped PEO-PPO-PEO triblocks, should be mentioned.

This paper describes the synthesis of PEO-PPO-PEO dimethacrylate derivatives obtained by the reaction of the OH-terminated PEO-PPO-PEO triblocks with methacryloyl chloride. The reactive macromonomers were then crosslinked by free radical polymerization in an aqueous medium and at physiological temperature. This work focuses on Pluronic F127 (12 600 Daltons, 70% w/w PEO), the most important member of this family. The characterization of the synthesized macromonomer and crosskinked systems was carried out by NMR, FTIR, GPC, and DSC. In addition, the temperature-dependent viscoelastic behavior of the hydrogels was analyzed by means of compression tests and rheological measurements and the microstructure of the lyophilized crosslinked sample was observed by environmental SEM. It is important to stress that Pluronic F127 has proved to be non-irritating when applied topically or intracutaneously, and elicited little irritation following intramuscular or intraperitoneal administration [22].

MATERIALS AND METHODS

Materials

The solvents were of analytical grade and were dried by adding molecular sieves 4A (BDH). Pluronic F127 was purchased from Sigma and dried at 120° C under vacuum for 2 h. Methacryloyl chloride was obtained from Aldrich and was distilled before use. Triethylamine (TEA) and sodium metabisulfite were supplied by Riedel–de-Haën and Aldrich, respectively, and both compounds were used as received.

Synthesis of PEO-PPO-PEO dimethacrylate

40.1 g (3.2 mmol) of Pluronic F127 was poured into a three-neck flask and dried as described above. The polymer was then dissolved in 75 ml of dry chloroform and the solution was cooled to 0° C in an ice bath. 2.63 g of TEA (26.3 mmol) was added. 2.65 g (26.3 mmol) of freshly distilled methacryloyl chloride was diluted in 20 ml of chloroform and added drop-wise for 2 h to the cooled mixture, under a dry nitrogen flow and magnetic stirring. Finally, the reaction was allowed to proceed for 24 h at room temperature. The crude product was dried under vacuum and resuspended in hot toluene (100 ml). The hot mixture was filtered in order to remove the triethylammonium hydrochloride salt and F127 dimethacrylate (F127 DMA) was precipitated in 400 ml of petroleum ether, 60–80°. The white solid product was filtered under vacuum; washed with several portions of petroleum ether, 40–60°; and dried under vacuum at room temperature.

Preparation of crosslinked Pluronic DMA gels

Gels were prepared by dissolving 3 g of F127 DMA in 12 ml of distilled water. 20 mg of ammonium persulfate (APS) was dissolved in 100 μl of water and added to the solution at low temperature and homogenized. Then 20 mg of sodium metabisulfite was dissolved in 100 μl of water, added to the solution and mixed thoroughly. Finally, the 20% (wt/wt) polymer system was incubated at 37° C for 24 h.

Characterization

Gel permeation chromatography (GPC). The average-molecular weights and polydispersity ($\bar{M}_w/\bar{M}_n$) were determined by GPC (Differential Separations Module Waters 2690 with refractometer detector Waters 410 and Millenium Chromatography Manager), using polystyrene standards between 472 and 360 000 Daltons.

Nuclear magnetic resonance (NMR) spectrometry. ^{1}H- and ^{13}C-NMR spectra were recorded in a Bruker 300 MHz NMR (spectrometer operating at 75.5 MHz for ^{13}C and 300 MHz for ^{1}H measurements). All spectra were obtained at room temperature from 15% (wt/v) $CDCl_3$ solutions.

Fourier transform infrared (FTIR) spectrometry. The characterization of the functional groups was carried out by FTIR analysis using a Nicolet Avatar 360 FTIR spectrometer. The samples were prepared by solvent casting from chloroform solutions, directly on sodium chloride crystals (Aldrich).

Viscosity measurements. The rheological behavior of the water solutions of F127 and F127 DMA before crosslinking was studied using a Brookfield Viscometer DV-II, with Bath/Circulator TC-500 and Wingather Software, using a T-F spin-

dle at 0.05 cycles per minute. The temperature was stabilized for at least 15 min before each measurement.

Rheological behavior of crosslinked systems. The crosslinked systems were analyzed by means of a penetration test [23, 24] and by rheometry. For the penetration measurement, a cylindrical probe (12 mm in diameter) was forced into a gel formed in a cylindrical container (30 mm in diameter and 10 mm in height) at 37° C. The crosshead displacement rate was 0.1 mm/min. The force measured, F, and the penetration distance (0.3 mm) were used to determine the relative viscosity. A minimum of five specimens were tested.

For the rheological analysis, an AR 1000 rheometer (TA Instruments), using parallel plates as the measurement geometry, was used. F127 DMA 20% crosslinked samples were allowed to swell in water at 37° C until equilibrium was achieved and then disc-shaped specimens (20 mm in diameter and 2.5 mm in thickness) were cut out. Frequency sweep and temperature ramp oscillation tests were performed. In the first one, a sinusoidal stress was applied to the sample over a range of frequencies (10^{-3} to 50 Hz) and the storage modulus (G'), loss modulus (G'') and phase angle (G''/G' or $\tan\delta$) were monitored. In the temperature ramp mode, the viscoelastic properties were measured as a function of the temperature and time, in the 5–50° C range at 5° C/min and an angular frequency of 1 Hz.

Swelling test. Rectangular specimens (12 mm × 7 mm × 3 mm) were cut out from crosslinked F127 DMA 20% (wt/wt) gels, dried until a constant weight was obtained, and then incubated in deionized water at different temperatures for various periods of time. The swelling degree (%) was calculated from the equation

$$\text{Swelling}(\%) = \frac{W_s - W_d}{W_d} \times 100, \tag{1}$$

where W_s is the sample weight after equilibrium swelling and W_d is the weight of the dry sample. The standard deviation of the results was 5%.

Thermal analysis. Thermal analysis was carried out by differential scanning calorimetry (DSC) (Mettler Toledo 822^e). The samples were sealed in 40 μl Al-crucible pans and their weight was kept between 18 and 22 mg. The gels were subjected to two consecutive runs: first, they were heated up from – 5° C to 50° C and then cooled down back to – 5° C, at 10° C/min heating or cooling rate. The enthalpy of micellisation was obtained from the area of the peak relative to the baseline.

Scanning electron microscopy. Samples were cut into thin slices, stored at – 24° C and lyophilized. The morphology was studied by means of environmental scanning electron microscopy (ESEM) (Philips XL-30).

RESULTS AND DISCUSSION

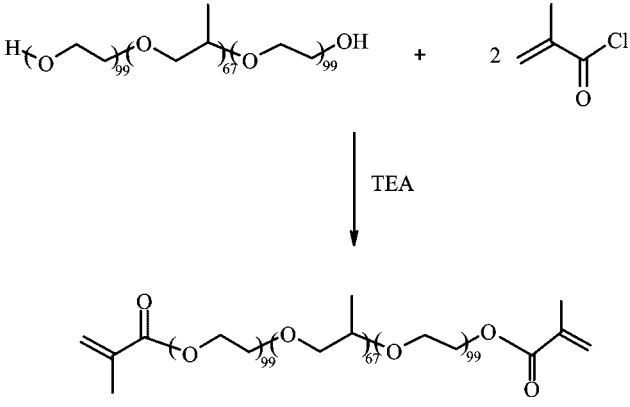
Synthesis and characterization of Pluronic F127 dimethacrylate (F127 DMA)

The functionalization of the OH-terminated PEO-PPO-PEO triblocks is shown in Scheme 1. The progress of the reaction was followed by FTIR, which showed the gradual appearance of weak bands at 1713 and 1635 cm^{-1}, corresponding to the carbonyl vibration of the ester group and to the vinyl double bond, respectively (see Fig. 1). Moreover, ^{1}H-NMR (Fig. 2) and ^{13}C-NMR spectra (not shown here) demonstrated the incorporation of the methacryloyl groups and the formation of dimethacrylate end-capped F127 triblocks. The hydrogen atoms belonging to the unsaturated bond appeared at 5.62 ppm (doublet, one proton) and 6.18 ppm (doublet, one proton). The binding of the methacryloyl moieties allowed the identification of the signal corresponding to the last methylene group of the PEO block, which was shifted downfield to 4.3 ppm (triplet, two protons) by the ester group generated. Thus, from the integration of the vinyl protons and the above-mentioned methylene group belonging to the PEO segment, an accurate quantification of the functionalization yield was achieved. The ratio indicated that two methacryloyl units were introduced per triblock molecule.

In addition, the average-molecular weight and polydispersity were monitored by GPC. The relative values obtained were $\bar{M}_n$ = 15 300 and $\bar{M}_w$ = 19 600 for F127, and $\bar{M}_n$ = 16 600 and $\bar{M}_w$ = 21 900 for F127 DMA. The polydispersity for both copolymers was approximately 1.3.

Rheological behavior of F127 and F127 DMA solutions

The viscosity of aqveous solutions of F127 and F127 DMA was determined, in order to study the influence of the new functional end-groups on the rheological behavior. Figure 3 shows the viscosity of F127 and F127 DMA 20% (w/w) solutions as a function of the temperature. The results obtained confirmed that the presence of the



Scheme 1. Functionalization of the F127 triblock copolymer to render α,ω-dimethacryloyl F127 (F127 DMA).

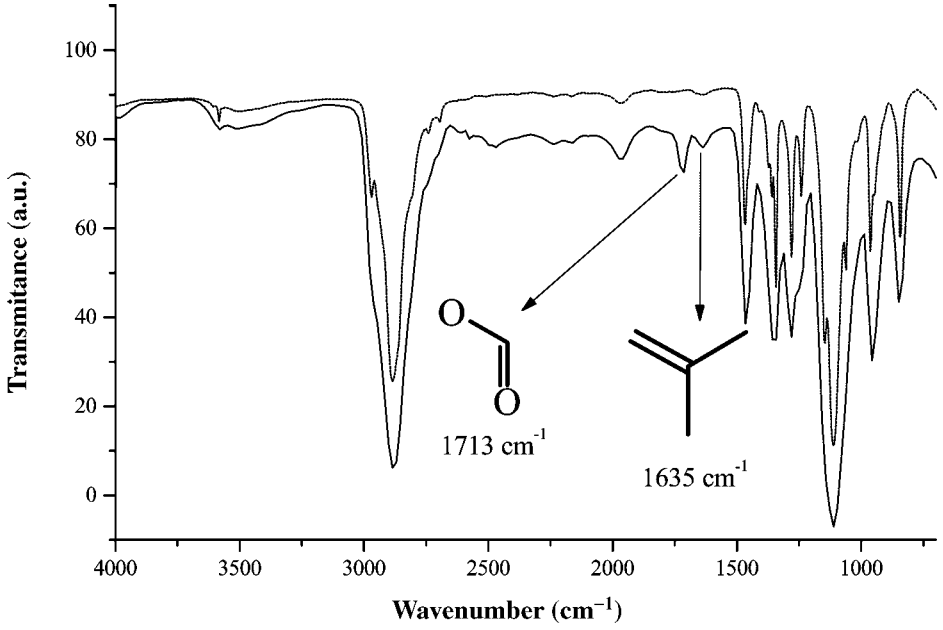


Figure 1. Infrared spectra of F127 (top spectrum) and F127 dimethacrylate (F127 DMA) (bottom spectrum).

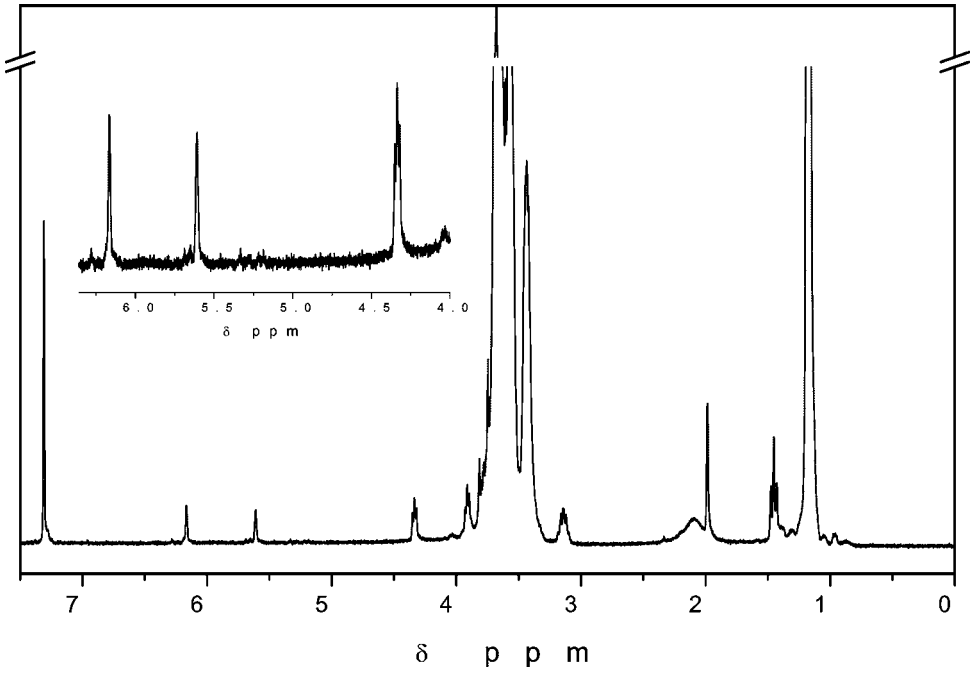


Figure 2. ^{1}H-NMR spectrum of the macromonomer F127 DMA.

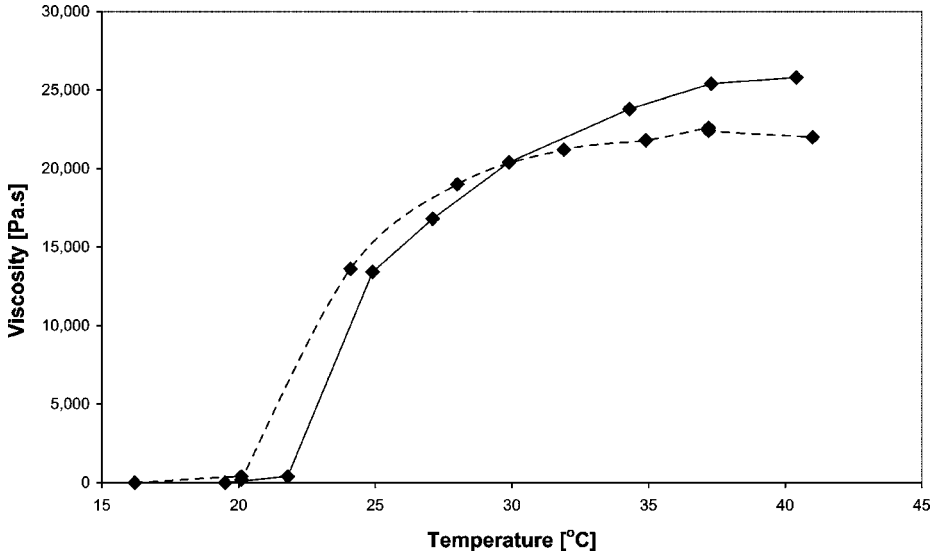


Figure 3. Evolution of viscosity as a function of the temperature for the F127 triblock copolymer before (- - -) and after (—) functionalization.

methacrylate end-groups did not affect the ability of the system to display reverse thermal gelation.

Free radical polymerization of F127 DMA aqueous solution at 37° C

In order to prepare crosslinked materials, the free radical polymerization of F127 DMA aqueous solutions was carried out using the ammonium persulfate/sodium metabisulfite initiator/catalyst redox system [25].

Characterization of the crosslinked polymeric structure

The uniaxial compression test is probably the simplest method to determine the elastic response of the sample in its gel state [26]. The compression modulus (E_c) can be obtained rather easily using the Mooney–Rivlin relationship (equation (2)), derived from continuum mechanics arguments and usually valid for small deformations:

$$\sigma = \frac{F}{A} = G \cdot \left(\lambda - \frac{1}{\lambda^2}\right), \tag{2}$$

where F is the measured force, A is the area, G is the modulus of elasticity (equal to one-third of the Young's compression modulus, E_c), σ is the true stress and λ is the deformation, expressed as the deformed length divided by the initial length (l/l_0).

As expected, crosslinking had a dramatic effect on the viscoelastic behavior of the system, as exemplified in Fig. 4, where the stress–deformation dependence is plotted as σ versus $(\lambda - \lambda^{-2})$. The compression modulus (E_c) was calculated as the

slope of the linear elastic region, multiplied by 3. While 20% (w/w) F127 DMA gels at 37° C showed a compression modulus of 142.5 ± 29.7 kPa, the crosslinked gel was three times stiffer, displaying an E_c value of 415.2 ± 45.7 kPa. The improved mechanical properties of the crosslinked system can also be illustrated by the different stress–deformation dependence that it exhibited. For example, at 2.5% deformation, the crosslinked hydrogel displayed stress values 22 times higher than those attained by uncrosslinked F127 DMA gel samples.

Table 1 summarizes the swelling data of crosslinked F127 at 25° C and 37° C, expressed as the swelling degree. In accordance with its reverse thermo-responsive behavior, the crosslinked specimen showed a clear decrease in its equilibrium water

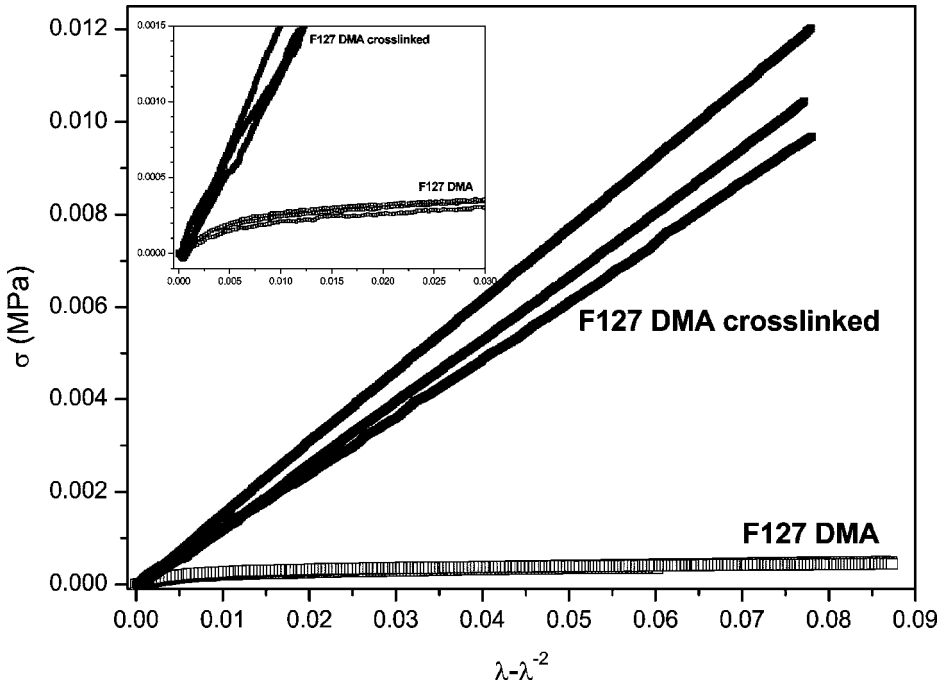


Figure 4. Stress–strain dependence of 20% (w/w) gel samples of F127 DMA before (open squares) and after (filled squares) crosslinking at 37° C; three specimens are plotted. Young's modulus in compression (E_c) was calculated from the slope in the linear region.

Table 1.
Swelling percent of crosslinked F127 DMA at 25° C and 37° C

Immersion time	Swelling percent	
	25° C	37° C
2 h	367	341
24 h	1160	713
2 days	1320	798
5 days	1630	771

absorption at higher temperatures. While the sample at 37° C achieved a swelling degree slightly below 800% after 4 days, the sample at room temperature attained a value of approximately 1600%. The gels prepared in this work behaved similarly to the hyaluronic acid/Pluronic thermo-sensitive composites developed by Kim and Park [27]. In their work, they described the ability of their Pluronic crosslinked systems to swell differently at different temperatures, with a clear release of water being observed at higher temperatures. They attributed this phenomenon to the molecular reorganization of the PEO-PPO-PEO segments between the crosslink linkages and to the entropy gained due to the release of water molecules from the hydrophobic segments.

In order to investigate the effect of crosslinking on micellisation, F127 DMA and crosslinked F127 DMA samples underwent a heating–cooling cycle. One would anticipate that the limited chain mobility of the crosslinked system would partially hinder micellisation, affecting both the temperature at which the transition takes place and its enthalpy content. Figure 5 presents the thermograms of F-127 DMA 20% before and after crosslinking. The findings revealed, though, that the difference between the micellisation process of the two systems pertained only to the energy involved in the formation of micelles. While the enthalpy value associated with the micellisation transition in F127 DMA was 4.7 J/g, the crosslinked gel showed a significantly lower value (3.1 J/g). On the other hand, the transition temperature of both systems remained unaffected, around 15° C. It should be stressed, though, that the ice-to-water transition was not observed, probably due to the cryoscopic

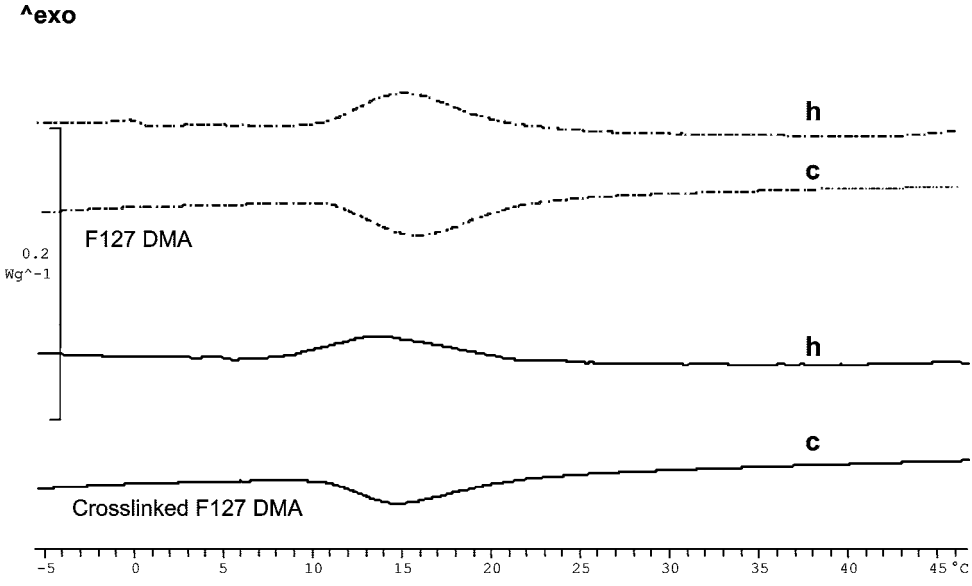


Figure 5. DSC thermograms of F127 DMA (- · - · -) and the crosslinked system (—). h and c represent heating and cooling curves, respectively.

decrease of the ice melting temperature. These results are in full accord with the data published by Booth and co-workers [28].

Regarding the rheological behavior, the linear viscoelastic range was determined by means of a simple stress amplitude or torque sweep test, with a fixed frequency of 1 Hz. The complex modulus (G^*) was measured as a function of the stress amplitude or the oscillatory torque, and the linear viscoelastic range was limited to the amplitude range for which G^* remained constant. From this preliminary experiment, a torque of 50 mN m was selected for the dynamic measurement, where the storage (G'), the loss modulus (G'') and tan δ were measured as a function of the temperature, at constant frequency (see Fig. 6).

The analysis of these curves provided further insight into the viscoelastic behavior of crosslinked F127 DMA systems. For the entire temperature range studied, the shear storage modulus was much higher than the loss modulus ($G' \gg G''$). This finding was indicative of a gel behavior [26, 29]. It is also worth noticing the temperature-dependent profile of tan δ. The presence of a peak at 35.1°C could be associated with the thermally induced deswelling of the system, produced by the release of water at higher temperatures.

In Fig. 7, the dynamic moduli (G' and G'') of these gels are plotted versus the angular frequency at 5°C and 37°C. The effect of the frequency on the storage modulus was negligible at frequencies lower than 10 rad/s (1.6 Hz) and a clear

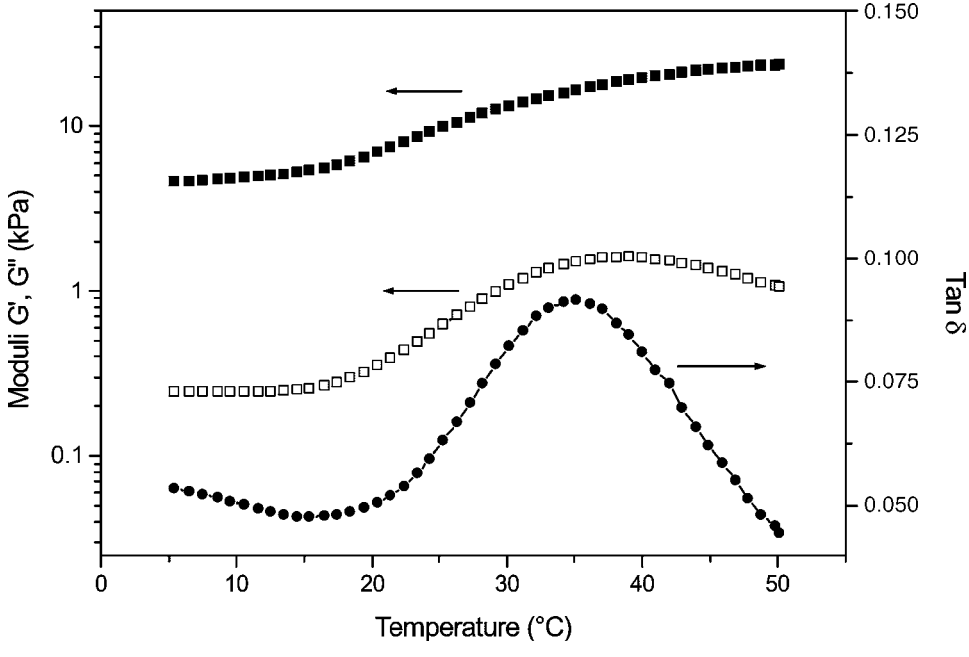


Figure 6. Viscoelastic behavior of the crosslinked system. Storage (G') (filled squares) and loss (G'') (open squares) moduli and tan δ circles of a 12% (w/w) hydrogel produced by the crosslinking reaction of F127 DMA, measured at 1 Hz, as a function of the temperature.

plateau was apparent in the rubber region. The loss modulus values measured at 37° C showed an increase in the low frequency range. This behavior can be associated with the long time of the test, which allowed the deswelling of the sample. On the other hand, at the same frequency, the loss modulus at 37° C was higher than that observed at 5° C. This finding is in agreement with the results shown in Fig. 6 and indicates that an increase in temperature leads to a shift from a more elastic to a more viscous response.

Networks obtained during the crosslinking process of high-molecular-weight polymers or prepared by chemically end-linking chains are, in general, far from ideal, since only a fraction of the polymer present contributes to the rubber modulus. Accordingly, the network consists of elastic chains, dangling or free ends, and a sol fraction with primary polymer molecules [30]. The study of the network structure in terms of its sol and network weight fractions was therefore of interest. Crosslinked F127 DMA samples were extracted in distilled water, in order to determine their sol fraction, w_s. The experimentally determined value of w_s was 0.12. This result clearly indicated that the network formation was not complete and that it contained a small amount of soluble polymer. This could be attributed to the relatively low concentration of the reactive dimethacrylate moieties in the crosslinking system, due to the fairly long F127 chains (12 600 Da).

In addition, it is well known that networks prepared by exhaustively reacting the functional groups have M_c values similar to M_n [31]. In the case of incompletely crosslinked networks, the average molecular weight between crosslinks, M_c, is

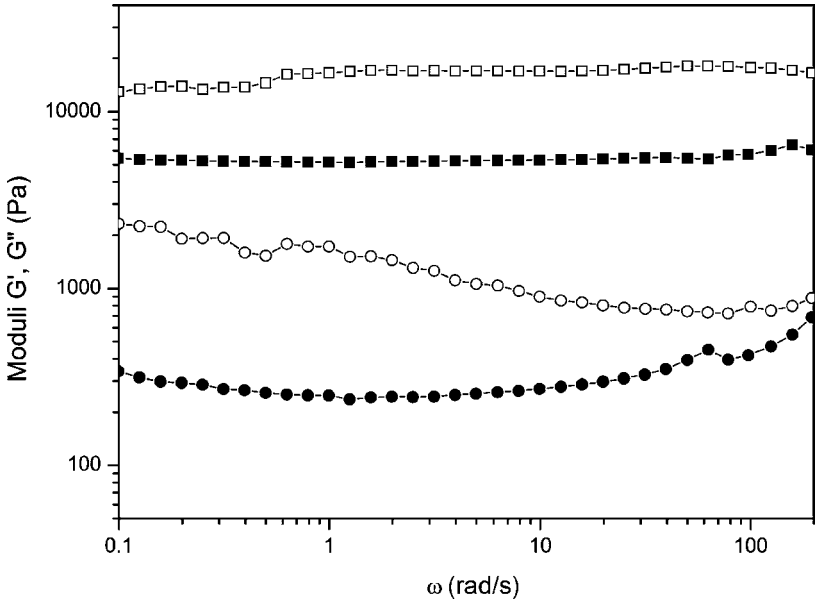


Figure 7. Dynamic moduli G' (squares) and G'' (circles) as a function of the angular frequency of a swelled hydrogel produced by the crosslinking reaction of F127 DMA, measured at 5° C (filled symbols) and 37° C (open symbols).

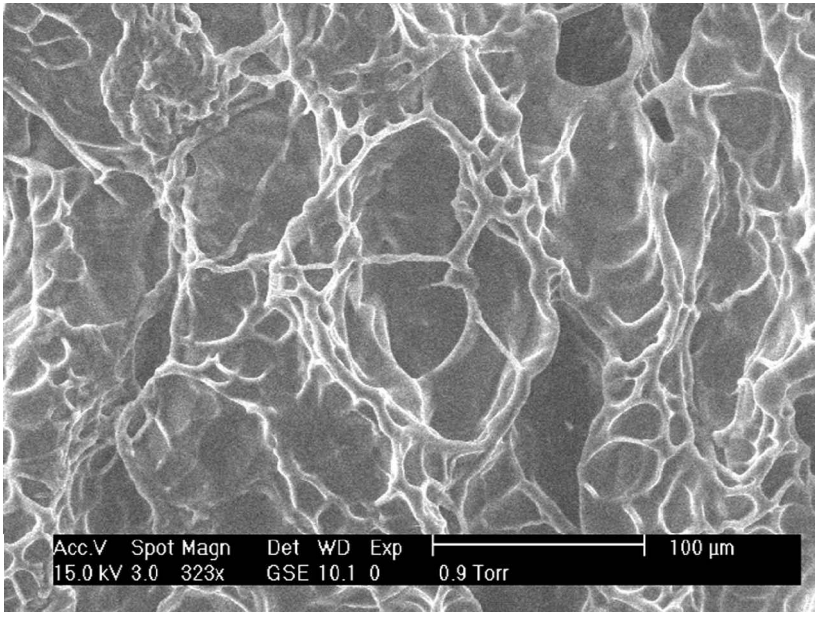


Figure 8. Environmental SEM micrograph of a lyophilized hydrogel.

higher than M_n, the average-number molecular weight of the chains prior to their crosslinking. The stress–strain data at a low deformation limit and application of the semi-empirical Mooney and Rivlin equation and the equations proposed by Mark and Llorente provided one way of estimating the M_c value in the network [31]. The M_c value calculated was slightly higher than M_n (17 400 and 16 600, respectively).

In order to gain further insight into their structure, crosslinked gels were lyophilized and studied by environmental SEM. Figure 8 shows micrographs of F127 DMA, where a highly porous, sponge-like network was apparent.

CONCLUSIONS

The present work has presented a rheological study of the modified PEO-PPO-PEO block copolymer Pluronic F127 before and after crosslinking by free radical polymerization in water medium and physiological conditions. The F127 dimethacrylate crosslinked gels displayed substantially improved physical properties, while retaining their initial reverse thermo-responsive behavior. Accordingly, they are expected to overcome the limitations exhibited by the uncrosslinked F127 triblocks related to the low residence times and represent a preferred alternative in the clinical setting. Degradation tests and drug release studies from both matrices, F127 DMA and crosslinked F127 DMA, are currently being conducted and will be published shortly.

Acknowledgements

A. Sosnik thanks the Levi Eshkol Fund, Ministry of Science, Israel (Grant No. 0328988) for support. We also thank David Gómez for the ESEM imaging.

REFERENCES

1. A. S. Hoffman, *J. Control. Release* **297**, 6 (1987).
2. R. Pelton, *Adv. Colloid Interface Sci.* **85**, 1 (2000).
3. N. A. Peppas, P. Bures, W. Leobandung and H. Ichikawa, *Eur. J. Pharm. Biopharm.* **50**, 27 (2000).
4. A. Carlsson, G. Karlstrom and B. Lindman, *Colloids Surfaces* **47**, 147 (1990).
5. R. Zana, W. Binanalimbele, N. Kamenka and B. Lindman, *J. Phys. Chem.* **96**, 5461 (1992).
6. R. Zana, N. Kamenka, L. Burgaud and B. Lindman, *J. Phys. Chem.* **98**, 6785 (1994).
7. B. Jeong, S. W. Kim and Y. H. Bae, *Adv. Drug Deliver. Rev*. **54**, 37 (2002).
8. J. W. Lee, F. Hua and D. S. Lee, *J. Control. Release* **73**, 315 (2001).
9. J. Z. Krezanoski, US Patent No. 4,188,373 (1980).
10. P. Alexandridis and T. A. Hatton, *Colloids Surfaces A* **96**, 1 (1995).
11. A. Hoffman, *Adv. Drug Deliver. Rev.* **54**, 3 (2002).
12. Y. Deng, G. Yu, C. Price and C. Booth, *J. Chem. Soc., Faraday Trans.* **88**, 1441 (1992).
13. A. Hoffman, *Artif. Organs* **19**, 458 (1995).
14. G. Wanka, H. Hoffmann and W. Ulbricht, *Macromolecules* **27**, 4145 (1994).
15. L. Yang, P. Alexandridis, D. C. Steyler, M. J. Kositza and J. F. Holzwarth, *Langmuir* **16**, 8555 (2000).
16. A. Steinleitner, H. Lambert, C. Kazensky and B. Cantor, *Obstet. Gynecol.* **77**, 48 (1991).
17. E. Esposito, Y. Carotta, A. Scabbia, L. Trombelli, P. D'Antona, E. Menegatti and C. Nastruzzi, *Int. J. Pharm.* **142**, 9 (1996).
18. M. Katakam, W. R. Ravis, D. L.Golden and A. K. Banga, *Int. J. Pharm.* **152**, 53 (1997).
19. B. S. Kim, J. S. Hrkach and R. Langer, *Biomaterials* **21**, 259 (2000).
20. J. A. Hubbell, C. P. Pathak, A. S. Sawhney; N. P. Desai and J. L. Hill, US Patent No. 6,306,922 (2001).
21. C. Pathak, S. P. Barman, C. M. Philbrook, A. S. Sawhney, A. J. Coury, L. Z. Avila and M. T. Kieras, US Patent No. 6,201,065 (2001).
22. L. Reeve, in: *Handbook of Biodegradable Polymers — Drug Targeting and Delivery*, A. Domb, J. Kost and D. Wiseman (Eds), Vol. 7, p. 63. Harwood Academic Publishers, Amsterdam (1997).
23. D. G. Oakenfull, N. S. Parker and R. I. Tanner, in: *Gums and Stabilisers for Food Industry* 4, G. O. Phillips, P. A. Williams and D. J. Wedlock (Eds), p. 231. IRL Press, Oxford (1988).
24. C. M. Gregson, S. E. Hill, J. R. Mitchell and J. Smewing, *Carbohydr. Polym.* **38**, 255 (1999).
25. A. Sawhney, D. A. Melanson, C. P. Pathak, J. A. Hubbell, L. Z. Avila, M. T. Kieras, S. D. Goodrich, S. P. Barman, A. J. Coury, R. S. Rudowsky and D. J. K. Weaver, US Patent No. 5,844,016 (1998).
26. J.-M. Guenet, in: *Thermoreversible Gelation of Polymers and Biopolymers*, p. 187. Academic Press, London (1992).
27. M. R. Kim and T. G. Park, *J. Control. Release* **80**, 69 (2002).
28. G. Yu, Y. Deng, S. Dalton, Q. Wang, D. Attwood, C. Price and C. Booth, *J. Chem. Soc., Faraday Trans.* **88**, 2537 (1992).
29. S. B. Ross-Murphy, *Polym. Gels Networks* **2**, 229 (1994).
30. K. te Nijenhuis, *Adv. Polym. Sci.* **130**, 8 (1997).
31. J. E. Mark, *Adv. Polym. Sci.* **44**, 5 (1982).