

Chain extension as a strategy for the development of improved reverse thermo-responsive polymers[†]

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Received 10 April 2007; Accepted 18 May 2007

The hypothesis that chain extension can be harnessed to the generation of improved reverse thermo-responsive polymers was tested by following two basic synthetic pathways: (1) the polymerization of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblocks using hexamethylene diisocyanate (HDI) as chain extender and (2) the covalent binding of poly(ethylene glycol) and poly(propylene glycol) chains, using phosgene as the connecting molecule. While in the former, the basic amphiphilic repeating unit is known for its own RTG behavior, the latter polymers consist of segments incapable of exhibiting a reverse thermal gelation (RTG) of their own. Dynamic light scattering (DLS) measurements revealed that the nanostructures formed by the chain extended polymers were markedly larger than those generated by PEO-PPO-PEO triblocks. While the size of Pluronic F127 micelles ranged from 15 to 20 nm, the higher molecular weight amphiphiles generated much larger nanostructures (20–400 nm). The chain extended polymers achieved much higher viscosities and their gels displayed enhanced long-term stability at 37°C. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: polymers; reverse thermo-responsiveness; rheological properties

INTRODUCTION

With the aim of minimizing the serious problems inherent to all fully open surgical procedures, increasing efforts are being devoted to develop improved, injectable, polymeric systems that would allow to conduct minimally invasive surgery. These materials are required to display low viscosity at insertion time, while a gel or solid consistency is developed *in situ*, later on. The “*in situ* generated implants” concept constitutes the basis of an especially innovative area that harnesses this behavior to the engineering of biomedical systems at their site of performance.

Various strategies were pursued in recent years to achieve this objective, such as the *in vivo* precipitation approach developed by Dunn *et al.*¹ and the more widely used *in situ* polymerization and/or crosslinking approach.^{2–5}

The term “thermo-responsive” refers to the ability of a polymeric system to achieve significant chemical, mechanical, or physical changes due to small temperature differentials. The “reverse thermo-responsive” phenomenon, usually known as reverse thermal gelation (RTG), is one of the most promising strategies for the development of injectable, *in vivo* generated biomedical systems. The water solutions of these materials display a low viscosity at ambient temperature and exhibit a sharp viscosity increase as temperature rises within

a narrow temperature interval, producing a semi-solid gel at body temperature. Since the mid-1990s, several polymers have been investigated, with much work focusing on poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblocks.^{6–9} Unfortunately, the viscosity increase achieved by these triblocks proved to be not large enough, resulting in systems displaying limited stability, poor mechanical properties, unacceptably high permeabilities and unsuitably short *in vivo* residence times. Despite their large clinical potential, these major shortcomings rendered these reverse thermo-responsive gels clearly inappropriate for most biomedical applications.

With the objective of overcoming these important limitations and substantially expanding the clinical applicability of RTG-displaying materials, our group has developed a series of new reverse thermo-responsive polymers, displaying superior rheological and mechanical properties. To this end, we followed two main avenues. The first focused on generating high molecular weight RTG-displaying polymers,^{10–13} while the second produced crosslinkable RTG-exhibiting polymers.^{14,15}

The present study goes one step further and reports the mechanical and rheological properties of new chain extended reverse thermo-responsive polymers, with the objective of harnessing their superior features to the *in situ* generation of biomedical systems. Numerous applications of these improved “smart” polymers are anticipated in various biomedical areas such as drug and gene delivery and tissue engineering.

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[†]This paper is presented as part of a special issue in memory of Professor Yair Avny.

EXPERIMENTAL

Materials

The solvents used were of analytical grade and were dried by adding molecular sieves 4A (BDH). The different triblocks used (EO₉₉-PO₆₇-EO₉₉, EO₁₂₅-PO₄₅-EO₁₂₅, EO₁₄-PO₃₀-EO₁₄, EO₁₁-PO₁₆-EO₁₁, and EO₉-PO₂₁-EO₉) were purchased from Sigma and dried at 120°C under vacuum for 3 hr, before use. Poly(ethylene glycol) and poly(propylene glycol) chains of various molecular weights were supplied by Aldrich and dried at 120°C under vacuum for 1 hr, before using. The phosgene chloroformic solution was prepared in our laboratory from 1,3,5-trioxane (Aldrich) and carbon tetrachloride (Frutarom), using aluminum chloride (Merck) as catalyst, following a previously described technique.¹⁶ Pyridine was purchased from BDH and was dried with molecular sieves 4A (BDH). Hexamethylene diisocyanate (HDI) and stannous 2-ethyl-hexanoate (SnOct₂, Sigma) catalyst were used as received.

Synthesis of the polymers

Syntheses of the polymers comprising different PEO-PPO-PEO triblocks as their basic repeating units, were carried out as described in detail elsewhere.¹⁰ The reaction conditions were fine tuned to optimize the polymerization of the different triblocks, depending on their respective molecular weight and the degree of polymerization (DP) targeted at. The one-pot synthesis of the random block poly(ether carbonate)s comprising poly(ethylene glycol) and poly(propylene glycol) segments coupled via phosgene, was carried out as described in detail elsewhere.¹⁵ Also for these polymers, the reaction conditions were optimized as required.

Characterization

Gel permeation chromatography (GPC)

The average molecular weights and polydispersity values ($\overline{M}_w/\overline{M}_n$) 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 Da.

Nuclear magnetic resonance spectroscopy (NMR)

¹H-NMR spectra were recorded in a Bruker 300 MHz NMR (300 MHz for ¹H measurements). All spectra were obtained at room temperature from 10% (wt/v) CDCl₃ solutions.

Infrared spectroscopy (FTIR)

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).

Dynamic light scattering (DLS)

The average hydrodynamic radius of the nanostructures formed in aqueous media was measured by DLS (Zetasizer 3000 HAS, Malvern Instruments, U.K.) using an external laser (Ar-laser, 488 nm, power 70 mW) in 4 ml polymethyl-

methacrylate disposable cuvettes. The particle size was taken as the mean value of three measurements.

Rheological measurements

The viscosity versus temperature behavior of the polymers and gels was studied using a Brookfield Viscometer DV-II, with Bath/Circulator TC-500 and Wingather Software, using a T-F spindle at 0.05 rpm. The temperature was stabilized for at least 7 min before each measurement. The storage modulus (*G'*) was determined using parallel plates HAAKE RheoScope-1 Optical rheometer. The samples were investigated over the 5–55°C, at 1°C/min heating rate and a constant angular frequency of 10 Hz.

RESULTS AND DISCUSSION

The working concept

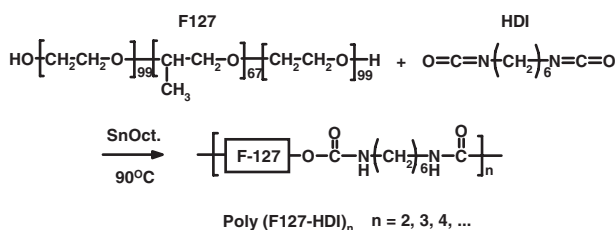
Injectable materials can be classified into two basic categories, according to their potential application: (i) as hydrophobic polymers that are able to perform physical or mechanical functions, where their load-bearing ability plays a key role and (ii) as highly hydrophilic, water-containing gels, characterized by their enhanced permeability to active biomolecules and cells. These polymers are especially suitable for the controlled release of hydrophilic macromolecules and in the tissue engineering field.

Acknowledging the need for the development of a new category of injectables that combines straightforward injectability, large water contents, and enhanced mechanical properties, this paper describes a family of novel reverse thermo-responsive polymers exhibiting superior RTG properties. In contrast to the well-known PEO-PPO-PEO triblocks' liquid crystalline organization based on self-assembly, our working concept aimed at developing amphiphilic polymers where basic repeating units are covalently bound along the polymeric backbone. By doing so, vicinity constraints are generated, new supramolecular architectures are formed, and multiblock copolymers displaying superior properties are produced.

Two basic synthetic avenues were followed: (i) polymerization of PEO-PPO-PEO triblocks using HDI as a chain extender, whereby poly(ether urethane)s were created and (ii) covalent binding of poly(ethylene glycol) and poly(propylene glycol) chains using phosgene, as a result of which poly(ether carbonate)s were formed. While in the former, the amphiphilic PEO-PPO-PEO repeating unit is known for its own reverse thermo-responsive behavior, the latter polymer consisted of hydrophilic and hydrophobic repeating segments, devoid of RTG of their own.

The chain extended polymers

Since it is the most important member of this family of materials, our work initially focused on the EO₉₉-PO₆₇-EO₉₉ triblock (MW 12,600; 70 wt% PEO) and capitalized on its own reverse thermo-responsiveness. The chain extended polymer was produced by reacting the triblock in an essentially 1:1 molar ratio with HDI, via the hydroxyl end groups of the former and the isocyanate moieties of the latter, as described in Scheme 1.



Scheme 1.

The occurrence of the chain extension reaction was conclusively demonstrated by GPC measurements which revealed the required increase in molecular weight. Furthermore, the progress of the chain extension reaction was monitored by observing (i) the gradual disappearance of the characteristic NCO band of the HDI molecule at 2272 cm^{-1} and (ii) the concurrent appearance and growth of the peak at 1732 cm^{-1} , belonging to the just formed urethane bonds.

As apparent from the plots shown in Fig. 1 generating a high molecular weight polymer resulted in polymers displaying remarkably enhanced rheological properties, this effect being stronger, the higher the DP. At 37°C , for example, a modest value of approximately 2000 was obtained for the basic $\text{EO}_{99}\text{-PO}_{67}\text{-EO}_{99}$ triblock (DP = 1), increasing to above 27,000 Pa for a high molecular weight polymer having a DP of 5.5.

Expectedly, the G' attained increased with concentration, shifting from around 2000 Pa for a rather diluted 9 wt% gel, up to above 22,000 for a 20 wt% concentration, in the case of a polymer with DP = 4.3. Furthermore, it is not only that the gels generated by the chain extended polymers are substantially sturdier, but also their minimal concentration for gellation is significantly lower, when compared to that of the $\text{EO}_{99}\text{-PO}_{67}\text{-EO}_{99}$ triblock. While the latter generates a gel only above 14.6 wt%, as determined in our laboratory, the chain extended polymers undergo a significant sol/gel transition at concentrations as low as 5.5 wt%.

It is also worth stressing that the nanostructures generated by these multiblock copolymers were much larger than those

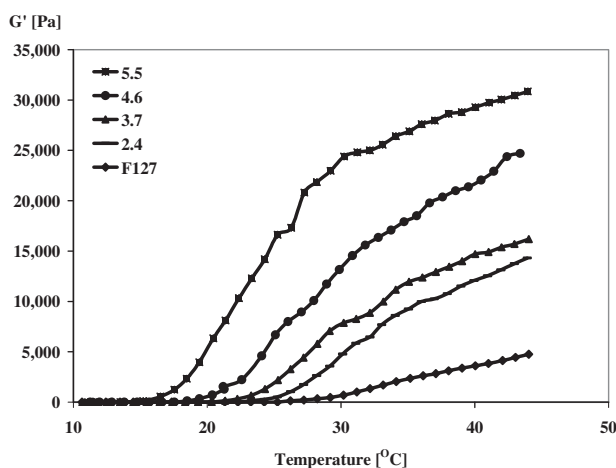


Figure 1. Rheological properties as a function of the degree of polymerization.

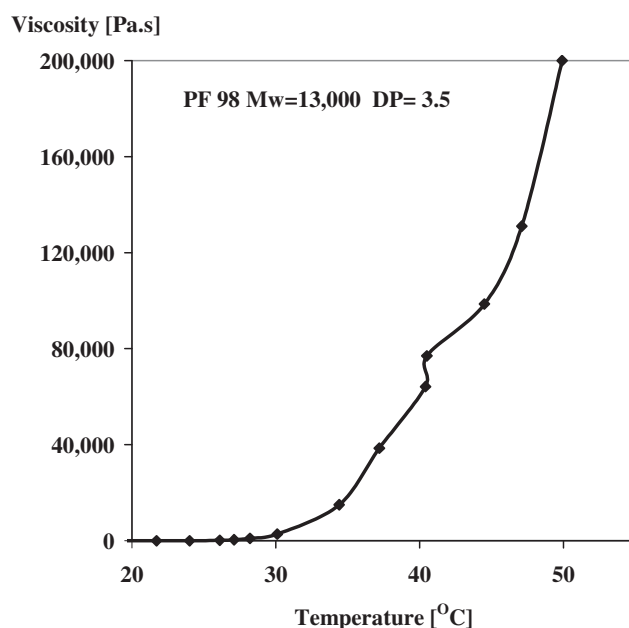


Figure 2. Viscosity versus temperature curve of PF 98.

formed by the PEO-PPO-PEO molecules (up to 400 nm for the former, as opposed to around 20 nm for the $\text{EO}_{99}\text{-PO}_{67}\text{-EO}_{99}$ triblock). It is reasonable to hypothesize that the large size of the supramolecular architecture generated by the chain extended polymers, plays a key role in enhancing their rheological properties. This issue is currently under investigation in our laboratory and will be reported separately.

Expectedly, the substantial effect of chain extension on the RTG phenomenon is not limited to the $\text{EO}_{99}\text{-PO}_{67}\text{-EO}_{99}$ triblock, with many other triblocks displaying a similar behavior. The temperature-dependent viscosity curve shown in Fig. 2 illustrates this behavior for a polymer based on the $\text{EO}_{125}\text{-PO}_{45}\text{-EO}_{125}$ triblock (MW 12,750) having an average DP value of 3.5. While the "monomeric" $\text{EO}_{125}\text{-PO}_{45}\text{-EO}_{125}$ triblock reaches a viscosity maximum value of around 12,000 Pa·s, its chain extended counterpart attains levels approximately 18 times higher.

While the two triblocks, $\text{EO}_{99}\text{-PO}_{67}\text{-EO}_{99}$ and $\text{EO}_{125}\text{-PO}_{45}\text{-EO}_{125}$ and their respective chain extended counterparts follow the same basic pattern, the sol/gel transition for the latter has shifted to a higher temperature due to its higher hydrophilicity. Also, low molecular weight triblocks, such as $\text{EO}_{14}\text{-PO}_{30}\text{-EO}_{14}$ (MW 2900) displaying a very limited reverse thermo-responsiveness, attain considerable viscosity levels, once polymerized. As shown in Fig. 3, a 20% water solution of a polymer comprising almost twelve $\text{EO}_{14}\text{-PO}_{30}\text{-EO}_{14}$ repeating units, displays a maximum viscosity of around 320 Pa·s. This is in clear contrast to the fairly low peak viscosity ($\sim 90\text{ Pa}\cdot\text{s}$) attained by a highly concentrated 60% solution of the "monomeric" triblock.

Following the same working concept that guided us when developing the polymers described above, we addressed the question whether materials lacking any RTG behavior can be converted in reverse thermo-responsive polymers by chain extending them. While in the polymers already studied, the amphiphilic PEO-PPO-PEO repeating units displayed their own, albeit minor reverse thermo-responsive behavior, this

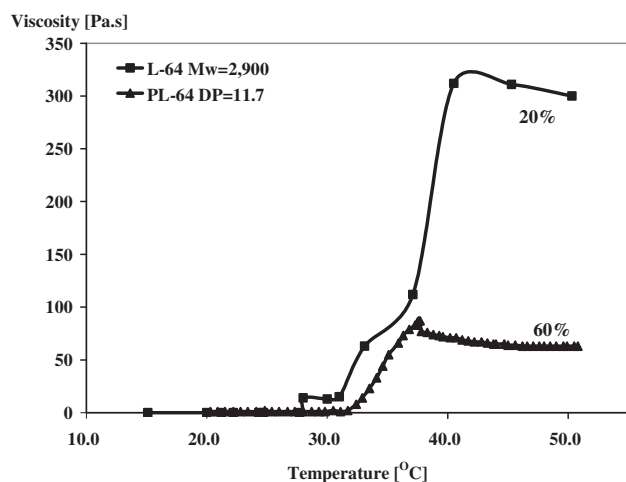


Figure 3. Viscosity versus temperature curves of the monomeric and the polymeric triblocks.

type of chain extended polymers consist of the following: (a) PEO-PPO-PEO triblocks which fail to display the RTG phenomenon or (b) hydrophilic and hydrophobic repeating segments, both of them devoid of any reverse thermo-responsiveness of their own.

The fact that triblocks that fail to exhibit any RTG behavior, regardless of their concentration and the temperature range studied, can grow to be reverse thermo-responsive polymers once chain extended, is especially noteworthy. This is unequivocally demonstrated in Fig. 4(a) and (b), for two low molecular weight triblocks: EO₉-PO₂₁-EO₉ and EO₁₁-PO₁₆-EO₁₁, both of them having a molecular weight of around 2000 Da.

Interestingly, the poly(ether urethane) formed by covalently binding 18 EO₉-PO₂₁-EO₉ triblocks, displayed the RTG phenomenon, as demonstrated by the sharp increase in its viscosity within a very narrow temperature interval (see Fig. 4a). Climbing markedly from extremely low viscosity levels, a 20% aqueous solution of this polymer gelled around 28°C, approaching its maximum viscosity of around 850 Pa·s at around 41°C, while at body temperature it attained a significant 650 Pa·s level. Additionally, the EO₁₁-PO₁₆-EO₁₁-based polymer having a DP=29, shows an abrupt gelation at around 30°C, attaining levels close to 800 Pa·s, as shown in Fig. 4(b). This is in striking contrast to the fact that the low molecular weight EO₁₁-PO₁₆-EO₁₁ triblock has no RTG behavior whatsoever.

The second type of polymers were engineered starting from building blocks lacking the RTG response, and these were copolymers comprising poly(ethylene glycol) and poly(propylene glycol) chains, using phosgene as the coupling molecule, as described in Scheme 2.

The molecular weight increase measured by GPC demonstrated the occurrence of the chain extension reaction. The progress of the reaction was followed by FTIR spectroscopy, which revealed the appearance and gradual increase of the peak at 1746 cm⁻¹, characteristic of the carbonate group generated by the reaction between phosgene and the hydroxy-capped poly(ethylene glycol) and poly(propylene glycol) chains. Concurrently, a steady decrease in the intensity of the band around 3400 cm⁻¹, due to the disappearance

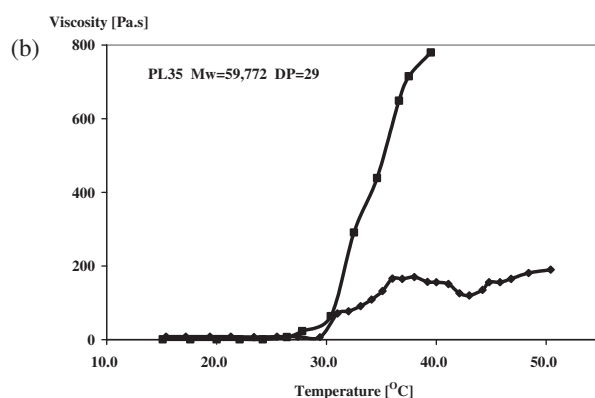
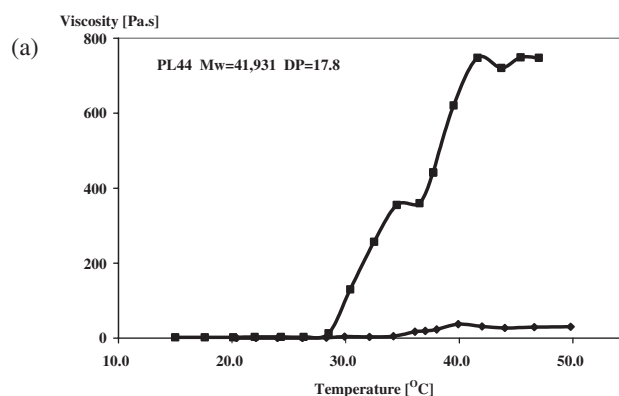
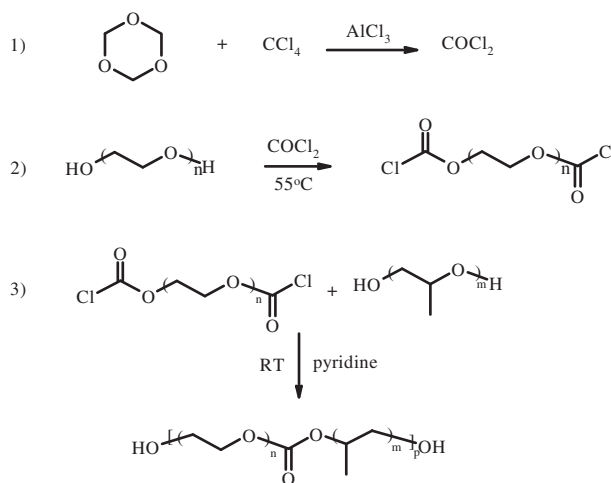


Figure 4. (a): Viscosity versus temperature curves of EO₉-PO₂₁-EO₉. (b): Viscosity versus temperature curves of EO₁₁-PO₁₆-EO₁₁.

of the OH end groups, was apparent. The composition of the PEO/PPO copolymers was determined using NMR spectroscopy, by taking the ratio of the peak assigned to PEO's methylene protons, at 3.65 ppm (singlet), and the peak at 1.12 ppm (triplet), due to the protons of PPO's methyl pendant groups.



Scheme 2. The use of phosgene as a coupling molecule for poly(ether carbonate) polymerization.

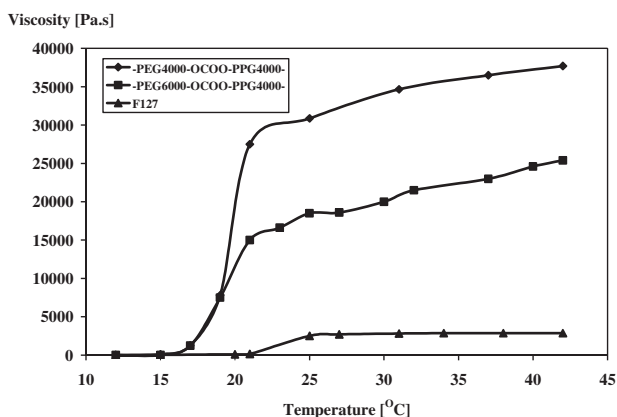


Figure 5. Viscosity versus temperature of 15% (w/w) solutions of two random poly(ether carbonate).

These new gels displayed at body temperature noticeably high viscosities, as exemplified in Fig. 5 for 15% (w/w) solutions of two random poly(ether carbonate) consisting of PEO and PPO blocks, reacted via phosgene. One polymer consisted of 4000 segments of PEO and PPO, while the second comprised 6000 PEO and 4000 PPO blocks.

As revealed by their viscosity versus temperature curves, the gels formed by these two poly(ether carbonate)s reached rather high viscosity levels (up to 38,000 Pa·s), whereas the gel formed by the EO₉₉-PO₆₇-EO₉₉ triblock (shown for comparison purposes) attained only a low 3000 Pa·s maximum viscosity.

Figure 6 shows the change in the size of the aggregates formed in aqueous medium by a poly(ether carbonate) comprising PEO 6000 and PPO 3000 segments, as a function of concentration. There are two important differences between the behavior of these chain extended polymers and that of a basic PEO-PPO-PEO triblock, as exemplified hereby for the EO₉₉-PO₆₇-EO₉₉ triblock. While the latter created micelles having a hydrodynamic radius of around 8–9 nm, this poly(ether carbonate) created much larger aggregates. Moreover, while the hydrodynamic radius of the micelles formed by the triblock remained constant as the

concentration rose, the size of the supramolecular assemblies formed by the poly(ether carbonate) increased in size with the increase in concentration in the aqueous medium. It is worth stressing that even at rather low concentrations (0.1 wt%), the size of the structures formed by this poly(ether carbonate) was remarkably large (around 90 nm), increasing further, up to 180 nm for a 1.0 wt% solution.

Another important feature of these gels pertains to their enhanced long-term stability, as shown in Fig. 7(a) and (b) which present the viscosity-over-time behavior of gels of the EO₉₉-PO₆₇-EO₉₉ triblock and two chain extended polymers having degrees of polymerization of 2.4 and 3.8, respectively.

It is apparent from the curves plotted in Fig. 7(a) that the gel formed by the triblock lost all its viscosity over an extremely short 3-day period, while both chain extended polymers displayed much improved stability. It could be argued, though, that the initial viscosity of the three gels is different and that, since this could affect the stability of the gel, a more relevant comparison would be the one done between gels having the same viscosity at time zero. This was achieved by significantly increasing the concentration of the triblock and decreasing that of the polymer with the highest DP value, while the polymer with DP = 2.4 was kept constant, whereas, at these different concentrations, all three gels had the same initial viscosity (around 82 Pa·s). As evident from Fig. 7(b), even when highly concentrated (32 wt%), the triblock formed a gel that deteriorated rapidly, losing all its viscosity after 20 days, whereas gels generated by the chain extended polymers exhibited much enhanced stability.

SUMMARY

This study demonstrated that chain extension constitutes a highly effective and versatile strategy for the production of improved reverse thermo-responsive polymers, displaying enhanced mechanical properties and superior long-term stability. The polymers investigated were produced via the following reactions: (i) the polymerization of PEO-PPO-PEO

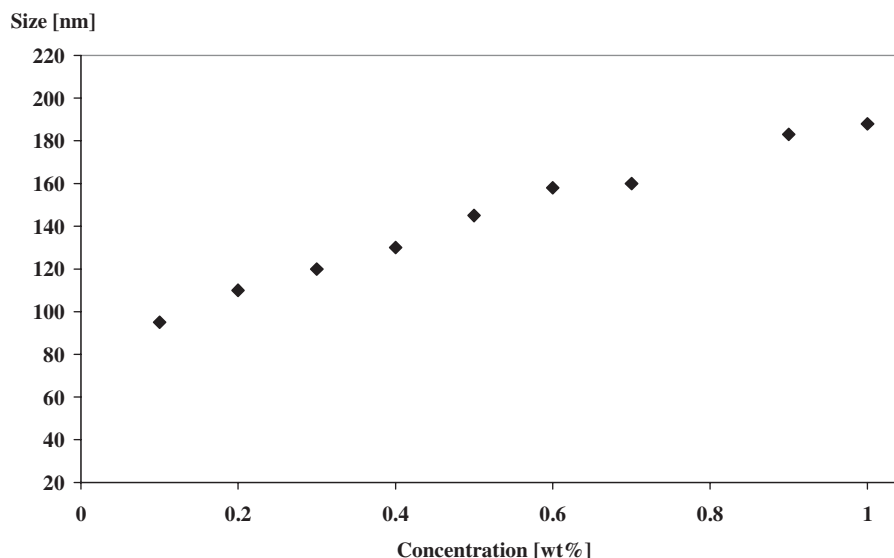


Figure 6. The change in the size of the aggregates as a function of concentration.

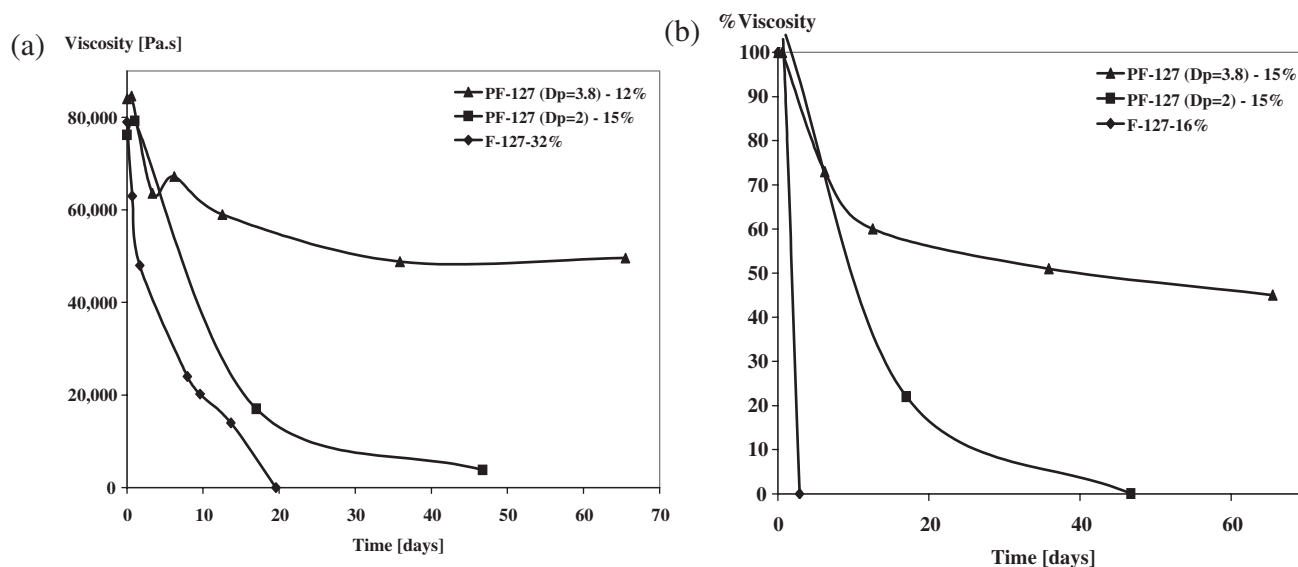


Figure 7. (a), 7(b): The viscosity-over-time behavior of gels of the $\text{EO}_{99}\text{-PO}_{67}\text{-EO}_{99}$ triblock and two chain extended polymers having degrees of polymerization of 2.4 and 3.8, respectively.

triblocks using HDI as chain extender and (ii) the covalent binding of poly(ethylene glycol) and poly(propylene glycol) chains, using phosgene as the connecting molecule. Furthermore, triblocks lacking any reverse thermo-responsiveness, started displaying the RTG phenomenon upon their chain extension. DLS findings revealed that the chain extended multiblock copolymers synthesized, created much larger nanostructures than the micelles formed by the triblocks.

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