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Novel reverse thermoresponsive injectable poly(ether carbonate)s

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The water solutions of polymers displaying reverse thermal gelation (RTG), such as poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblocks, exhibit a pronounced viscosity increase as temperature rises, within a very narrow temperature interval. Unfortunately, the viscosity increase attained by these solutions is not large enough, resulting in systems displaying limited stability and short residence times. This paper introduces a new family of reverse thermoresponsive alternating $[A\text{-}B]_n$ block copolymers, comprising poly(ethylene oxide) (PEO), and poly(propylene oxide) (PPO) chains, using phosgene as the molecule connecting both components. The effect of various compositional and structural parameters on both the C_i (minimal gelation concentration) and T_i (minimal gelation temperature) of these systems was investigated. The copolymers were characterized by GPC, ^{1}H-NMR, FT-IR, and DSC and the rheological behavior of the water solutions was studied using a Brookfield viscometer. The water systems were also studied by dynamic light scattering (DLS) and fluorescence spectroscopy. The copolymers developed exhibited clearly superior rheological properties, when compared to existing RTG-displaying PEO–PPO–PEO triblocks. For example, while the viscosity of a 15% water solution of the commercially available Pluronic F-127 achieved 5000 Pa.s, at 37 °C, poly(ether carbonate) water solutions (15%) attained viscosities between 25 000 and 150 000 Pa.s.

1. Introduction

The development of *injectable polymers*, suitable to be implanted in the human body without requiring a surgical procedure, has triggered much attention in recent years. Among them, the development of temperature-sensitive sol–gel systems represents one of the most promising strategies [1–3]. These materials combine low viscosity at the injection stage, with a gel or semisolid consistency being developed *in situ*, as the water solutions heat up to 37 °C after injection. Water solutions of polymers that exhibit this phenomenon, are known as reverse thermosensitive polymers and they display reverse thermal gelation (RTG).

The syringability of these materials is their most essential advantage, since it allows their introduction into the body using minimally invasive techniques. Furthermore, their low viscosity and substantial flowability at insertion time, enable them to reach and fill spaces otherwise unaccessible, as well as to achieve improved conformability and enhanced attachment to the tissues at the implantation site. Clearly, biodegradability is yet another important requirement of these materials.

Poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblocks, commercially known as Pluronics or Poloxamers [4, 5], are one of the most important and best investigated families of RTG-displaying materials [6–10]. Several mechanisms have been proposed as driving forces for their reverse thermal gelation behavior. Raising and Attwood [11] attributed the gel transition to intrinsic changes in the micellar properties. Vadnere and coworkers [12] explained the gelation in terms of entropic gain of the system, due to the breakdown of water molecules locally ordered around the hydrophobic segments (PPO) of the copolymer chain, when the system is heated. Other researchers assigned the RTG phenomenon to the formation of a three-dimensional network [8, 13]. Recently, several reports in the literature showed that the system becomes a gel because of a hard-sphere crystallization process, when the system approaches the critical volume value of 0.53 [14, 15].

Unfortunately, the viscosity increase achieved by the solutions of these PEO–PPO–PEO triblocks is not large enough, resulting in systems displaying limited stability and short residence times. These restrictions render these systems clearly unsuitable for most biomedical applications [16–18].

This paper introduces a new family of reverse thermoresponsive polymers, generated by copolymerizing hydrophilic poly(ethylene glycol) (PEG) chains and hydrophobic poly(propylene glycol) (PPG) segments, using phosgene as the molecule connecting both components. The choice of phosgene as the bifunctional coupling molecule allowed the selective reaction of the two components, in order to generate alternating $[A\text{-}B]_n$ block copolymers. The hydrophilicity and hydrophobicity of both segments, the molecular weight of each of the basic constituents and the degree of polymerization attained, played a fundamental role in determining not only the viscosity value achieved by the gel but also largely affected their C_i (minimal gelation concentration) and T_i (minimal gelation temperature).

2. Materials and methods

In general, the solvents used were of analytical grade and were dried adding molecular sieves 4A (BDH). Different poly(ethylene glycol) (PEG3400, PEG4000, and PEG6000) and poly(propylene glycol) (PPG3000 and PPG4000) chains were supplied by Aldrich and dried at 120 °C under vacuum for 1 h, 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. Pyridine was purchased from BDH. The synthesis was carried out following a two-step reaction, in order to obtain alternating $[A\text{-}B]_n$ copolymers.

2.1. The synthesis

The synthesis was carried out by a two-step reaction. The first step was the PEG dichloroformate synthesis at 60 °C [19], followed by the reaction between the PEG derivative and the PPG block [20], to produce the final block copolymer.

2.1.1. Preparation of phosgene solution in chloroform

The phosgene was generated by reacting 1,3,5 trioxane with carbon tetrachloride using aluminum trichloride as the catalyst, according to Kheyfets *et al.* [21]. The phosgene vapors were bubbled in weighed chloroform and the phosgene concentration (w/w) was calculated by weight difference (kept between 9 and 11%). Due to phosgene's high toxicity, the gas was handled with extreme care and all the work was conducted under a suitable hood.

2.1.2. Poly(ethylene glycol) 6000 dichloroformate synthesis

30.3 g (5 mmol) of dried PEG6000 (molecular weight 6000) were poured in a 250 ml flask and diluted in 50 ml dried chloroform. 22.0 g of chloroformic solution of phosgene 9% w/w (100% molar excess to PEG) were added to the PEG and the mixture was allowed to react at 60 °C for 4 h with magnetic stirring and condenser in order to avoid solvent and phosgene evaporation. The reaction flask was connected to a NaOH trap (20% w/w solution in water/ethanol 1 : 1) in order to trap the phosgene that could be evaporate during the reaction. Once the reaction was finished, the temperature was allowed to reach room temperature and the excess of phosgene was eliminated by vacuum. The same molar ratio was used for all the copolymers synthesized.

2.1.3. Chain extension reaction

15.2 g (5 mmol) of dry PPG3000 (molecular weight 3000) were added to the C1CO–PEG6000–COC1 obtained in Section 2.1.2, at room temperature. The mixture was cooled to 5 °C in an ice bath and 6.3 g pyridine (diluted in 20 ml chloroform) were added dropwise over a 15 min period. Then, the temperature was allowed to rise back to room temperature and the reaction was continued for 45 min. After that, the temperature was risen to 35 °C and the reaction was continued for one additional hour. The polymer produced was separated from the reaction mixture by adding it to 600 ml petroleum ether 40–60. The lower phase of the two-phase system formed was separated and dried at room temperature. Finally, the polymer was washed repeatedly with portions of petroleum ether and dried. A light yellow, brittle, and water soluble powder was obtained. The same molar ratio between the reagents was kept for the different block copolymers synthesized.

The PEO/PPO ratio in the different copolymers was determined by ^{1}H-NMR Bruker 300 MHz, using calibration curves based on PEG/PPG blends. The solvent used was chloroform-*d* ($DCCl_3$).

The characterization of the functional groups was carried out by FT-IR analysis using a Nicolet 510 FT-IR spectrometer. The samples were prepared by solvent casting from chloroform solutions, directly on sodium chloride crystals (Aldrich).

Thermal analysis was carried out by differential scanning calorimetry (DSC) (Mettler TA-400). The samples were sealed in 40 μl Al-crucible pans and their weight was kept between 9 and 11 mg. The polymers were subjected to three consecutive DSC runs: first, they were heated up to 80 °C, then cooled down back to − 100 °C and finally they were heated up to 80 °C. All runs were conducted at 5 °C/min heating or cooling rates. The crystallinity is directly proportional to the heat of fusion, ΔH_f. By integrating the normalized area of PEO's melting endotherm, determining its heat of fusion and rationing it to that of the respective PEG homopolymer, the relative crystallinity of the PEO segments, was assessed. This relative degree of crystallinity will be denoted by C_r. The value of ΔH_{f} for PEO homopolymer is 197 J/g.

The molecular weight, molecular weight distribution, and polydispersity (M_w/M_n) were determined by Gel Permeation Chromatography (Differential Separations Module Waters 2690 with Refractometer Detector Waters 410 and Millenium Chromatography Manager), using polystyrene standards between 472 and 360 000 Dalton.

The rheological behavior of the water solutions 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 15 min before each measurement.

The size of the microstructures present in the solutions was measured by dynamic light scattering (Malvern Zetasizer 3000HSA) with external laser (488 nm) in 4 ml polymethylmethacrylate disposable cuvettes. The solutions were prepared in miliQ water and were filtered by 0.45 μm Teflon filters.

The critical micellar concentration (CMC) was determined by fluorescence spectroscopy (LS-5 Perkin Elmer), using a pyrene probe The excitation wavelength was 336 nm, the emission was measured at 380 nm and the final pyrene concentration was in the 10^{-7} (molar) order, according to the technique in Bohorquez *et al.* [22]. The emission spectrum of pyrene consists of five primary bands, normally referred to as I_1–I_5, moving from shorter to longer wavelengths. The ratio of the intensity of the I_1 and I_3 peaks (I_1/I_3) is strongly affected by the polarity of the microenvironment the pyrene molecule is in, the ratio increasing as the polarity of the medium increases.

3. Results and discussion

3.1. Copolymer synthesis

The family of reverse thermoresponsive polymers introduced hereby was generated by copolymerizing segments which, separately, do not display the RTG phenomenon. The use of phosgene as the coupling molecule connecting between the hydrophilic and hydrophobic blocks along the copolymeric backbone, allowed to bind the two types of ''monomeric'' units, in an alternate mode. That, by capitalizing on the selective reactivity displayed by the two functional groups of phosgene and the easy elimination of the coupling agent excess by vacuum [19].

The block copolymers developed have the following general structure: $[–A–E–B–]_p$, where A is the hydrophilic block of the system (PEO), B is the hydrophobic component of the molecule (PPO), E is a bifunctional molecule (phosgene) and *p* is the degree of polymerization. Whereas various coupling agents were used, the present article focuses on phosgene (ClCOCl). The synthesis was carried out by a two-step reaction and is schematically described in Fig. 1. The first step was the synthesis of the PEG dichloroformate [19], followed by the reaction between the PEG derivative and the PPG chain [20], to produce the final block copolymer.

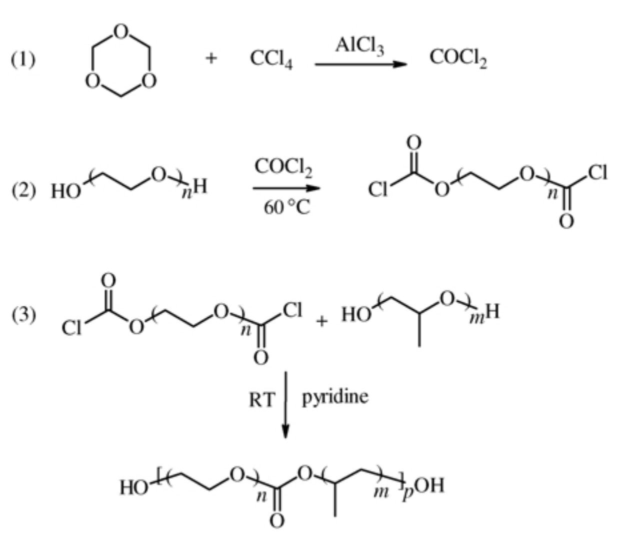

Figure 1 Synthesis and structure of the poly(ether carbonate)s. (1) Phosgene generation and chloroform solution preparation. (2) Synthesis of the PEG dichloroformate. (3) Chain extension reaction between the PEG derivative and PPG segment.

TABLE I Representative molecular weights of the synthesized poly(ether carbonate)s

Polymer	PEG (wt %)	M_n	M_w	M_w/M_n
Poly[PEG6000–COO–PPG3000]$_p$	78	28 100	44 000	1.56
Poly[PEG6000–COO–PPG4000]$_p$	70	28 700	44 000	1.53
Poly[PEG4000–COO–PPG4000]$_p$	56	25 500	39 000	1.53
Poly[PEG3400–COO–PPG4000]$_p$	49	29 200	39 500	1.35

3.2. Monitoring the polymerization

The growth of the copolymeric chain was followed by gel permeation chromatography and by FT-IR spectroscopy. Table I presents the molecular weight data of several copolymers synthesized, comprising various PEO and PPO segments.

The progress of the reaction was also studied by observing both the gradual disappearance of the C=O vibration of the PEG chloroformate, at 1777 cm^{-1}, and the concomitant appearance and growth of the peak at 1746 cm^{-1}, characteristic of the carbonate group, belonging to the final block copolymer (see Fig. 2).

3.3. The backbone composition

The ratio between the PEO and PPO blocks (% w/w) was determined by ^{1}H-NMR spectroscopy and calculated from calibration curves built using accurately weighed blends containing different ratios of the two components. The peaks used were that 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 (see Fig. 3).

3.4. Thermal properties

The thermal properties of the polymers were analyzed by differential scanning calorimetry. The change in PEG's segmental length affected the thermal properties of the polymers. The thermograms displayed in Fig. 4 clearly show a gradual increase in PEG's melting point.

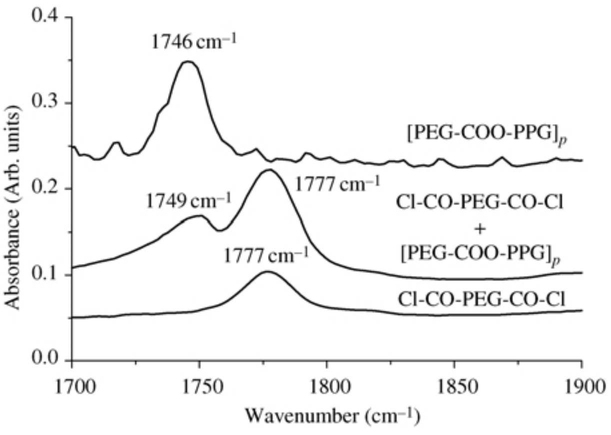

Figure 2 Characteristic carbonyl group vibration band at FT-IR.

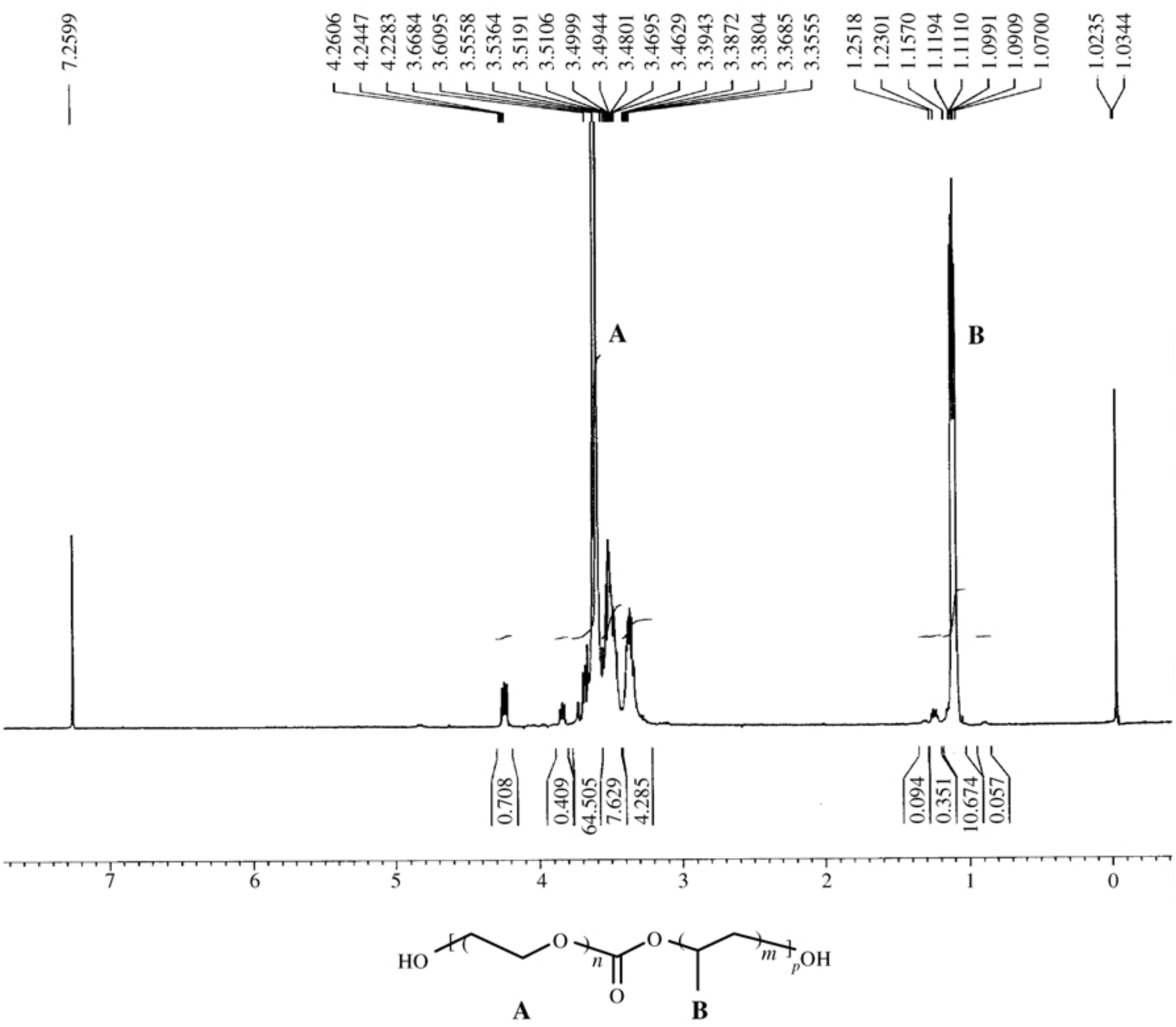

Figure 3 The backbone composition determined by ^{1}H-NMR.

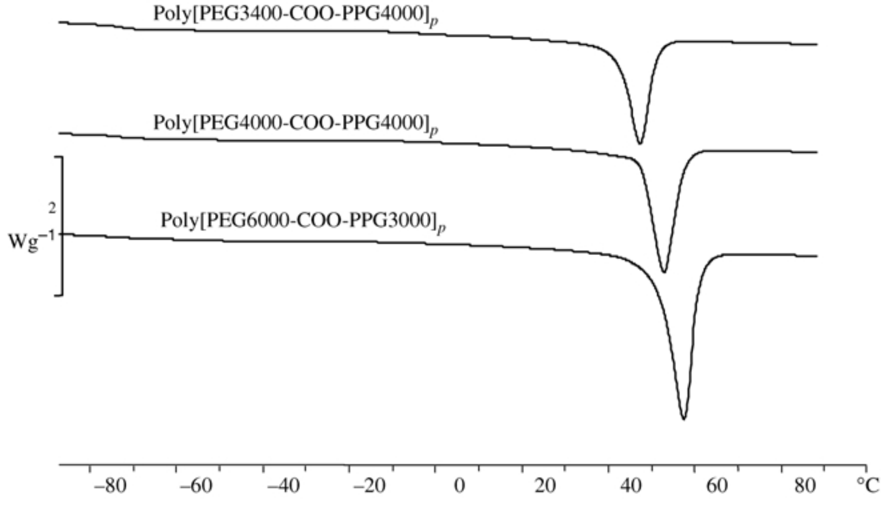

Figure 4 Thermal analysis of three poly(ether carbonate)s.

Furthermore, the enthalpy of fusion of the PEO segments rised as their molecular weight increased, with ΔH_f values shifting from 114 J/g, for Poly[PEG3400–COO–PPG4000]$_p$, 129 J/g for Poly[PEG4000–COO–PPG4000]$_p$ and 138 J/g for Poly[PEG6000–COO–PPG3000]$_p$, indicating the increase in the degree of crystallinity achieved by the material (see Table II). It is also clear that the PEO segments of the different copolymers were able to crystallize to a significant extent, as reflected by the high relative crystallinity (C_r) levels they attained. That, due to the limited constraints imposed by the extremely flexible PPO chains, on the crystallizable PEO segments.

TABLE II Thermal properties of copolymers

Polymer	PEG (wt %)	T_c[°C]	T_m[°C]	ΔH[J/g$_{PEG}$]	C_r[%]
Poly[PEG6000–COO–PPG3000]$_p$	78	33	54	138	70
Poly[PEG4000–COO–PPG4000]$_p$	56	30	50	129	65
Poly[PEG3400–COO–PPG4000]$_p$	49	22	45	114	58

TABLE III The critical micellar concentration at three different temperatures

Polymer	PEG (wt %)	CMC$_{10\,°C}$	CMC$_{17\,°C}$	CMC$_{37\,°C}$
Poly[PEG6000–COO–PPG3000]$_p$	78	0.07	0.03	0.005
Poly[PEG6000–COO–PPG4000]$_p$	70	0.04	0.01	0.004
Poly[PEG4000–COO–PPG4000]$_p$	56	0.04	0.003	0.002

TABLE IV Aggregates size as determined by DLS

Polymer	PEG (wt %)	Size 25 °C [nm]	Size 30 °C [nm]
Poly[PEG6000–COO–PPG3000]$_p$	78	101	82
Poly[PEG4000–COO–PPG4000]$_p$	56	89	74

3.5. Water solutions study

3.5.1. Critical micellar concentration

The critical micellar concentration was determined using a pyrene probe, following Pandit's technique [22]. It is apparent, therefore, that this technique provides information about CMC, since the (I_1/I_3) ratio will be large before micelles are formed, decreasing sharply upon micellization. Table III shows the CMC results for three of the polymers at 10, 17, and 37 °C, respectively. There are two factors affecting the CMC findings displayed. In first place, there is a clear decrease in CMC, as temperature rises. It is also clear that CMC values decreased as PPO content increased due to the higher hydrophobicity of the material.

3.5.2. Aggregate size

The covalent binding of the hydrophobic PPO blocks and hydrophilic PEO segments, generated high molecular weight alternating amphiphilic copolymers. The size of the microstructures formed in water, at concentrations above the CMC, was measured by dynamic light scattering. Table IV presents the average size of the aggregates created by two copolymers, Poly[PEG6000–COO–PPG3000]$_p$ and Poly[PEG4000–COO-PPG4000]$_p$, at 25 and 30 °C. It is apparent from the data presented that the microstructures generated by the poly(ether carbonate) chains are much larger (74–101 nm range) than that created by the PEO-PPO-PEO triblocks (around 15 nm).

3.5.3. Rheological behavior

As stressed before, the hydrophilic/hydrophobic balance of the copolymer and its molecular weight affect dramatically the rheological behavior of the different water systems. As an example, Fig. 5 presents the rheological behavior of an alternating poly(ether carbonate) based on PEO6000 and PPO4000 segments (PEO 70% and PPO 30% in weight), having a molecular weight of 28 700 (Mn). The viscosity vs. temperature behavior of 15% w/w water solutions (at 37 °C) of this copolymer is presented and compared with that of the commercially available Pluronic F-127 (12 600 Dalton), having the same PEO/PPO ratio. The pronounced difference in the rheological behavior is apparent. While the poly(ether carbonate) [PEG6000–OCOO–PPG4000]$_p$ attained a 25 000 Pa.s viscosity level, Pluronic F-127 achieved only a modest 5000 Pa.s value.

Aiming at assessing the influence of the hydrophilic/hydrophobic balance along the copolymeric chain, the

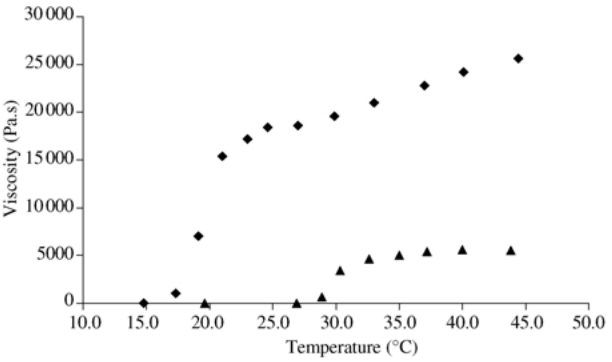

Figure 5 Rheological behavior of Poly[PEG6000–COO–PPG4000]$_p$ (◆), in comparison with the Pluronic F-127 (▲), in 15 wt % water solutions.

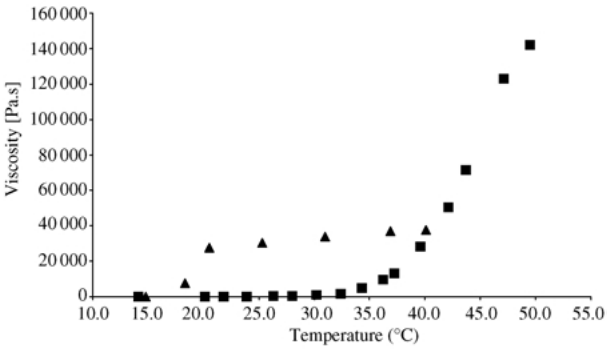

Figure 6 The rheological behavior of Poly[PEG6000–COO–PPG3000]$_p$ (■) and Poly[PEG4000–COO–PPG4000]$_p$ (▲).

rheological behavior of copolymers containing different PEO/PPO ratios, was studied. Fig. 6 compares two polymers having very similar MW (around 26 000 Dalton), differing only in their PEO/PPO ratio. Expectedly, the more hydrophilic material exhibited higher T_i and viscosity values.

4. Conclusions

The synthesis and characterization of the different copolymers were reported and some of the parameters affecting the behavior of the polymer in water were discussed. This novel group of RTG-displaying polymers exhibited clearly superior rheological properties, when compared to those of PEO/PPO/PEO triblocks. Furthermore, the new materials created much larger microstructures in the aqueous medium, when compared to the existing triblocks. It can be surmised that the properties of these copolymers, will also render them with enhanced long term stability. It is additionally expected that due to their higher viscosities at 37 °C, these novel gels will perform better as injectable matrices for slow release of bioactive molecules. These issues are currently under investigation and will be published separately.

Acknowledgments

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