THERMOSENSITIVE BRANCHED BLOCK COPOLYMERS OF POLY(ETHYLENE GLYCOL) AND COPOLYACRYLATES OF OLIGO(ETHYLENE GLYCOL)S : SYNTHESIS AND THERMAL GELATION PROPERTIES OF AQUEOUS SOLUTIONS

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Au fost sintetizaţi prin polimerizare radicalică cu transfer de atom bloc copolimeri ramificaţi cu lanţ central de poli(etilen glicol) de diferite lungimi si blocuri laterale reprezentate de copolimeri ai acrilatului de di(etilen glicol) etil eter cu oligo(etilen glicol) metil eter acrilat distribuiţi statistic (cu masa moleculară de aproximativ 480 Da). Comportamentul termosensibil al bloc copolimerilor sintetizaţi a fost studiat prin reometrie dinamică şi prin metoda inversiei eprubetei, pe soluţii apoase 20%. Rezultatele au arătat că atât temperaturile de asociere şi de gelifiere cât şi proprietaţile reologice au depins de masele moleculare ale blocurilor de polimer. Au fost efectuate teste de eliberare in vitro a 5-fluorouracilului, demonstrându-se capacitatea acestor bloc copolimeri de a forma sisteme injectabile cu eliberare lentă a medicamentelor.

Branched block copolymers with poly(ethylene glycol) middle chain of different lengths and side arms represented by random copolymers of di(ethylene glycol) ethyl ether acrylate and oligo(ethylene glycol) methyl ether acrylate (with a molecular weight of about 480 Da) were synthesized by atom transfer radical polymerization. The thermogelation behavior of the block copolymers in 20 wt% aqueous solutions was investigated by both dynamic rheometry and tube inversion method. The results showed that both association and gelation temperatures, as well as the rheological properties depended on the molecular weights of the polymer blocks. In vitro 5-fluorouracil release tests demonstrated the ability of the synthesized block copolymers to form injectable systems with controlled drug release.

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1. Introduction

Hydrogels are 3D hydrophilic polymer networks, physically or chemically crosslinked, able to retain large amounts of water or biological fluids [1,2,3]. Due to their ability to absorb water and their potential biocompatibility, hydrogels display a wide variety of biomedical applications in tissue engineering for the repairing and regeneration of certain tissues and organs, as controlled drug delivery systems, as matrices for cells encapsulation, etc. [4,5,6]. The dissolution of the hydrophilic polymer chains is prevented by crosslinks made by covalent bonds in permanent hydrogels (chemically crosslinked), or by physical interactions (molecular entanglement and/or secondary forces, like ionic or hydrogen bonds) in the case of reversible hydrogels (physically crosslinked) [5]. An increasing interest in physically crosslinked hydrogels was observed within the last years, the main reason being the lack of the crosslinking agents, which may negatively affect the integrity of the substance to be entrapped (proteins, cells, etc.), and which are often toxic compounds that have to be removed from the gels before use [2].

An unique characteristic of the hydrogels, which made them being called "smart' materials or stimuli responsive materials is their ability to suffer a phase transition in response to chemical stimuli (pH, ionic strength, chemical substances) or physical stimuli (temperature, magnetic or electric field) in the environmental media [4,7]. Among the sensitive/responsive hydrogels, the thermoresponsive ones are intensely studied because temperature is an effective and easy to apply stimulus, making them useful in many applications. At the transition temperature, the permanent hydrogels change their swelling behavior/ratio (e.g. abrupt increase or decrease of the gel volume in water), while the reversible hydrogels pass from the sol phase defined like a flowing fluid into the non-flowing gel phase [8]. The reversible thermoresponsive hydrogels are divided into three categories, depending on how their sol-gel transition occurs : 1) hydrogels with a low critical solution temperature (LCST), i.e. the polymer is in a soluble state when temperature is below the LCST, and it becomes hydrophobic and insoluble at temperatures above LCST ; 2) hydrogels with an upper critical solution temperature (UCST) when gel formation occurs following a chilling process (e.g. gelatin) ; 3) hydrogels which pass successively, at different temperatures, through sol-gel-sol transitions (e.g. poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers) [1,9-11].

Among the polymers forming thermoresponsive hydrogels, the most studied are those displaying LCST: poly(N-isopropylacrylamide), poly(N,N-diethylacrylamide), poly(N-vinylcaprolactam), poly(methyl vinyl ether), poly(propylene glycol), hydroxypropylcellulose, etc, as well as their copolymers. In biomedical applications, a LCST value close to human body temperature is needed, requirement that can be met by adjusting the hydrophilic monomer-hydrophobic monomer ratio within the copolymer. A very useful application of these hydrogels is represented by injectable hydrogels, which are solutions at room temperature or below, but after injecting them into the human body by means of a syringe they switch to a crosslinked gel state [12-14]. These represent a support for low invasive treatment methods, allowing to include drugs or cells in polymer solutions and release them after the physical crosslinking in the human body.

Recently, a new class of thermoresponsive polymers for biomedical applications, made mostly of oligo(ethylene glycol) biocompatible segments, was described. Among these, the methacrylates of various oligo(ethylene glycol)s, which are easily polymerizable by controlled radical polymerization techniques (ATRP, RAFT), have been the most studied [15-19]. Statistical copolymers denoted P(MEO_2MA-co-OEGMA), where MEO_2MA is 2-(2-methoxyethoxy)ethyl methacrylate ; OEGMA is oligo(ethylene glycol) methyl ether methacrylate [15,16], triblock copolymers denoted P(MEO_2MA-co-OEGMA)-b-PEG-b-P(MEO_2MA-co-OEGMA) and 4-arm star block copolymers denoted sPEG-b-P(MEO_2MA-co-OEGMA) (where sPEG represents PEG with 4 arms) [18,19] were synthesized. They displayed thermoreversible properties in aqueous solutions.

A less studied category is represented by the oligo(ethylene glycol) acrylates copolymers. To the best of our knowledge, there are only 2 studies in literature concerning the copolymerization of oligo(ethylene glycol) methyl ether acrylate with di(ethylene glycol) ethyl ether acrylate, either by reversible addition-fragmentation chain transfer polymerization (RAFT) [20], or by atom transfer radical polymerization (ATRP) [21]. The obtained copolymers exhibit a thermogelation behavior, with tunable LCST over the range of 15-90°C, which can be adjusted by varying the comonomer composition. These characteristics and, in addition, the particularity of being composed of the same biocompatible material (i.e. ethylene oxide units) inspired us to synthesize new copolymers with a poly(ethylene glycol) middle chain with different lengths and 4 side arms represented by the random copolymers of di(ethylene glycol) ethyl ether acrylate (DEGA) with oligo(ethylene glycol) methyl ether acrylate (OEGA, $M_n \approx 480$ Da). We selected this type of structure as a branched structure is preferred in biomedical applications (e.g. injectable solutions) in comparison with a linear structure, because the former possess a smaller hydrodynamic radius for the same

molecular weight, allowing an easier elimination of the polymer from the human body via the kidneys [22]. The synthesized copolymers were characterized by ^{1}H-NMR and gel permeation chromatography. The thermogelation behavior in aqueous medium was studied by dynamic rheometry and inversion tube method. The controlled drug release ability of the hydrogels obtained by the gelation of 20 wt% polymer aqueous solution was also studied "in vitro", in PBS solution (pH=7.4) at 37ºC. 5-Fluorouracil, a drug with anti-cancer activity, was chosen as the model drug.

2. Experimental part

2.1. Materials

α,ω-Dihidroxy PEG with approximate molecular weights of 2000 atomic mass units (Da) (PEG_{2000}, from Fluka), 4000 Da (PEG_{4000}, from Fluka) and 6000 Da (PEG_{6000}, from Scharlau) were used as received. Their hydroxyl numbers, determined by the acetylation method, were 54.55, 27.99 and 19,23 mg KOH/g, respectively, corresponding to number average molecular weights (M_n) of 2060 Da, 4010 Da and 5850 Da, respectively, calculated considering 2 hydroxyl groups for each PEG macromolecule. The monomers di(ethylene glycol) ethyl ether acrylate (DEGA, Aldrich 90%) and oligo(ethylene glycol) methyl ether acrylate (OEGA, M_n about 480 Da, Aldrich) were purified to remove the inhibitor. DEGA was purified by passing through a short basic Al_2O_3 column, prior to use. To purify OEGA, the monomer was first diluted with diethyl ether and then passed through a basic Al_2O_3 column. The obtained solution was filtered and the solvent was completely removed in a rotary evaporator. The purified monomer was stored in the freezer, at - 20ºC. Copper (I) bromide (CuBr, Fluka, 98%) was purified by stirring with glacial acetic acid overnight, filtered, washed with anhydrous ethanol and dried under vacuum. Toluene, 2,2'-bipyridyl (bipy, from Aldrich, 99%), N,N-dimethylformamide (DMF, from Fluka, >99%) and methylene chloride (from Chimopar) were used as received.

The phosphate buffered saline solution (PBS, pH = 7.4) was prepared by dissolving 8 g NaCl (from Sigma Aldrich), 3.6 g $Na_2HPO_4 \cdot 12\ H_2O$ (from Reactivul), 0.2 g KCl (from Reactivul) and 0.24 g KH_2PO_4 (from Fluka) in 1 L of distilled water. The solution pH was verified by means of a Consort NV pH-meter

The tetrafunctional macroinitiators α,ω-bis[2,3-di(2-bromo propionyloxy) propyl] PEG_x ($TBPEG_x$) were obtained from tetrahydroxy PEG_x and 2-bromopropionyl bromide (Aldrich, 97%), in benzene, by a similar procedure to the one previously described [23].

2.2. Synthesis of the $[P(DEGA\text{-}co\text{-}OEGA)]_2\text{-}PEG_x\text{-}[P(DEGA\text{-}co\text{-}OEGA)]_2$ block copolymers

The block copolymers synthesis was carried out in a 20 mL round bottom flask which was charged with : the purified monomers (the corresponding amount for each sample is presented in Table 1), the calculated amount of macroinitiator $TBPEG_x$ and 1.5 mL toluene. The first number in the sample codes in Table 1 indicates the approximate molecular weight of the middle PEG block (i.e. 2 stands for 2 kDa, 4 stands for 4 kDa and 6 stands for 6 kDa), while the second number is indicative for the target polymerization degree per arm. For example, TO4-75 is the code for the copolymer with PEG_{4000} as the central block and a target polymerization degree of 75 for each block arm.

After the macroinitiator dissolution, the resulting homogeneous solution was bubbled with nitrogen for 80 min, in order to remove oxygen. Separately, the catalyst was prepared in a 5-mL one neck conical bottom flask fitted with a side arm, by adding the calculated amounts of CuBr and bipy, (macroinitiator Br end groups : CuBr : bipy = 1:1:3 mole ratio). The flask was sealed with a rubber septum and cycled four times between vacuum and nitrogen to remove oxygen. Then, by means of a degassed syringe, 1.5 mL of degassed toluene were added and the mixture was stirred at room temperature for about 10 min. After the catalytic complex formed, the heterogeneous brown catalyst solution was transferred via a degassed syringe in the polymerization flask, and the reaction mixture was allowed to polymerize under stirring, at 90ºC, for 21 h. At the end of the polymerization period, the flask was opened and the catalyst was exposed to air, to oxidize the catalytic complex and stop the reaction. The viscous reaction mixture was diluted with methylene chloride and passed through a column filled with neutral alumina to remove the catalyst. The resulting solution was filtered on a 0.2 μm PTFE membrane, yielding a clear yellowish filtrate. After the complete methylene chloride removal under vacuum by means of a rotary evaporator, the obtained viscous residue was diluted with 15 mL DMF and then concentrated under vacuum, in order to remove toluene. All these evaporation procedures were performed at room temperature in the presence of hydroquinone to avoid the polymerization of the unreacted monomer. The polymer solution was then dialyzed against water (Roth membrane, Zellu Trans, molecular weight cut-off 4000-6000), for 5 days, changing the water daily. After dialysis, the solution was filtered with suction, on a 0.45 μm polypropylene membrane, and water was removed in a rotary evaporator, under vacuum. The last traces of water were removed by azeotropic distillation with ethanol, resulting a viscous yellow liquid. The global monomer conversion, determined gravimetrically was around 80% in all the cases (Table 1).

Table 1

Polymerization conditions

Sample	$DP_{n,block}$ [a]	DEGA (g)	OEGA (g)	$TBPEG_x$ (g)	Conversion %
TO2-50	50	2,48	0,33	0,19	84,3
TO4-50	50	1,24	0,16	0,17	81,5
TO4-75	75	3,73	0,49	0,34	84,2
TO4-100	100	4,97	0,66	0,34	79,5
TO6-50	50	2,48	0,33	0,51	84,4

[a] target degree of polymerization for the copolyacrylate block with $[DEGA]_0/[OEGA]_0 = 19/1$

2.3. Characterizations

^{1}H-NMR spectra were recorded on a 300 MHz Varian Gemini 300 BB spectrometer, with deuterated chloroform as a solvent. The molecular weights and molecular weights distributions were measured by gel permeation chromatography, on an Agilent Technologies 1200 series instrument, with a PLgel Mixed-C (300x7,5 mm) column and an Agilent 1200 differential refractometer, in DMF, at 23ºC and a flow rate of 1 mL/min. Polystyrene standards were employed for calibration. The rheological measurements were carried out on 20 wt% aqueous solutions on a Kinexus Pro (Malvern Instruments, U.K.) rheometer by employing parallel plates of 20 mm diameter with 0.5 mm gap, in oscillating mode. A solvent trap was used to prevent water evaporation.

The gelation temperature (T_{gel}) was determined by the tube inversion method [24], on 20 wt % polymer aqueous solutions (1 mL), introduced in glass tubes with 10 mm diameter. The tubes were kept for 15 min at constant temperature (±0.5°C) in a chilling/heating dry plate (Torrey Pines Scientific, Inc, U.S.A.) prior to inverting the tube. The experiment was carried out with gradual heating, at 1ºC intervals, from 20°C to 70°C. Before switching to the next temperature, the samples were cooled down in an ice-water bath, and homogenized. The temperature at which the polymer solution did not flow when the tube was inverted was considered T_{gel}. The temperature at which the syneresis of the already formed gel (T_{syn}) occurred was determined visually by the same method.

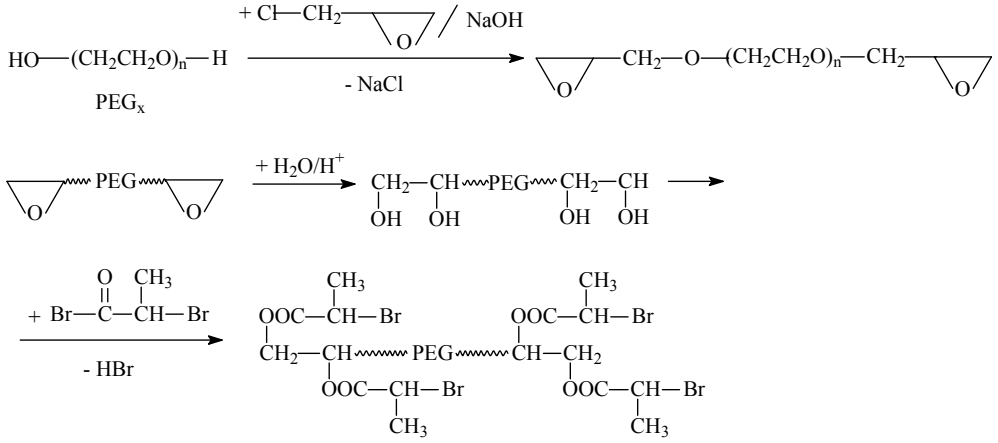
For the controlled drug release tests, about 0.2 g of polymer were weighed in a glass vial, followed by the addition of around 8 mg 5-fluorouracil (5-FU, Sigma Aldrich, 99%) and the calculated amount of PBS (pH = 7.4) to obtain a 20 wt% solution. After dissolution, the solutions were transferred into 50 mL polypropylene tubes and placed in a chilling/heating dry plate at 37±0.5ºC. After about 30 minutes, 30 mL PBS pre-heated at the same temperature were added to the formed gel and stirring was started (orbital stirring, 200 rpm). Samples were withdrawn (1 mL solution) at predetermined time intervals, diluted with PBS to 10 mL and analyzed by UV-VIS spectroscopy (UV-3600 Shimadzu instrument),

at 265 nm wavelength, using quartz cells with a light path of 10 mm. After each sample withdrawal, 1 mL fresh PBS at 37ºC was added, in order to preserve a constant volume of the release medium.

3. Results and discussion

3.1. Synthesis of the initiators

The tetrafunctional PEG macroinitiators synthesis was accomplished starting from the corresponding oligomers with OH end groups (PEG_x). The macroinitiators were obtained by a three-step procedure, similar with the one previously described [23] (Scheme 1).



Scheme 1

The functionality of the *α,ω*-bis[2,3-di(2-bromopropionyloxy) propyl] PEG_x macroinitiators ($TBPEG_x$) was determined from the ^{1}H-NMR spectra (Fig. 1), by comparing the areas of the peaks corresponding to the methyl protons (CH-CH_3) (δ = 1.82 ppm) and oxyethylene unit protons (CH_2CH_2O) (δ = 3.65 ppm). Thereby, the macroinitiators functionalities were: 3.8 for $TBPEG_{2000}$, 3.7 for $TBPEG_{4000}$ and 3.5 in the case of $TBPEG_{6000}$.

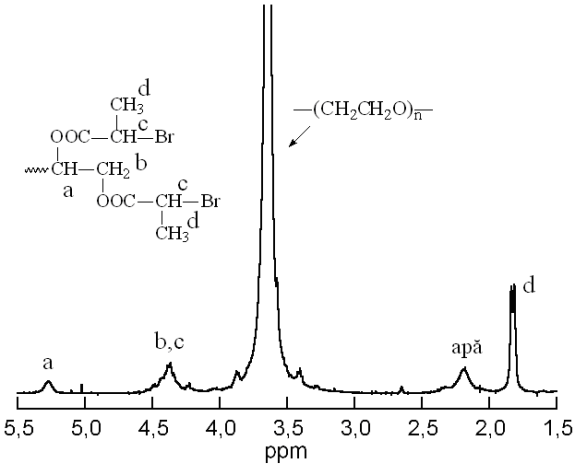
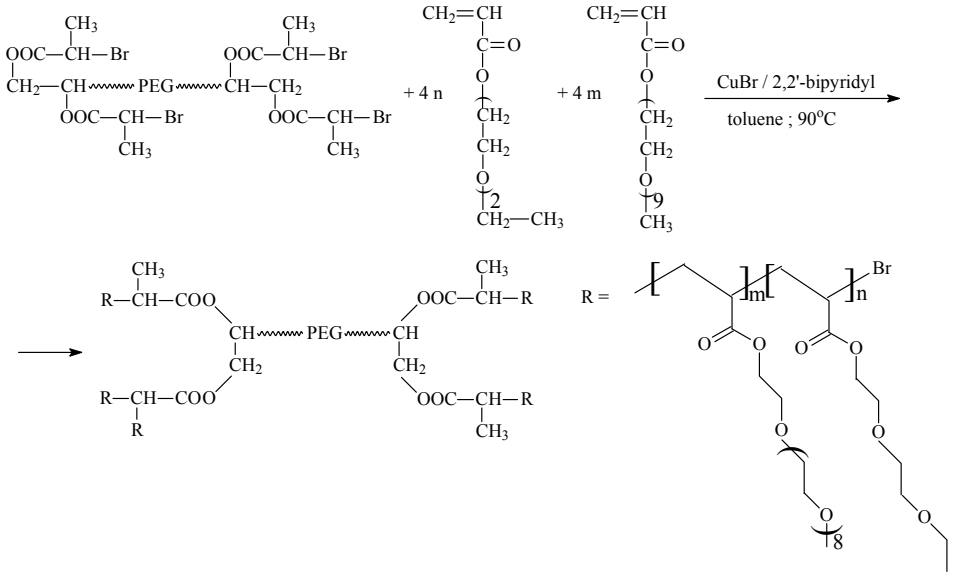


Fig. 1. ^{1}H-NMR spectrum of the TBPEG$_{2000}$ macroinitiator

3.2. Block copolymers synthesis

The block copolymers [P(DEGA-co-OEGA)]$_2$-PEG$_x$-[P(DEGA-co-OEGA)]$_2$ were synthesized by the copolymerization of DEGA and OEGA initiated by the tetrabromo PEG macroinitiators and catalyzed by CuBr/bipy in 3 ml toluene. The reaction occurs through an ATRP mechanism (Scheme 2).



Scheme 2

The obtained block copolymers were characterized by ^{1}H-NMR spectrometry and gel permeation chromatography. The ^{1}H-NMR spectra of the branched block copolymers synthesized displayed the characteristic peaks of both PEG block and monomer units of DEGA and OEGA (Fig. 2). By comparing the areas of the methyl peak (–CH_3) of DEGA segments at δ = 1,17 ppm, and methyl group peak of OEGA units (δ = 3,34 ppm) (Fig. 2), the composition of the statistical copolymer block was determined (Table 2). The obtained values were in good agreement with the comonomers initial molar ratio in the reaction mixture, as expected [20,21].

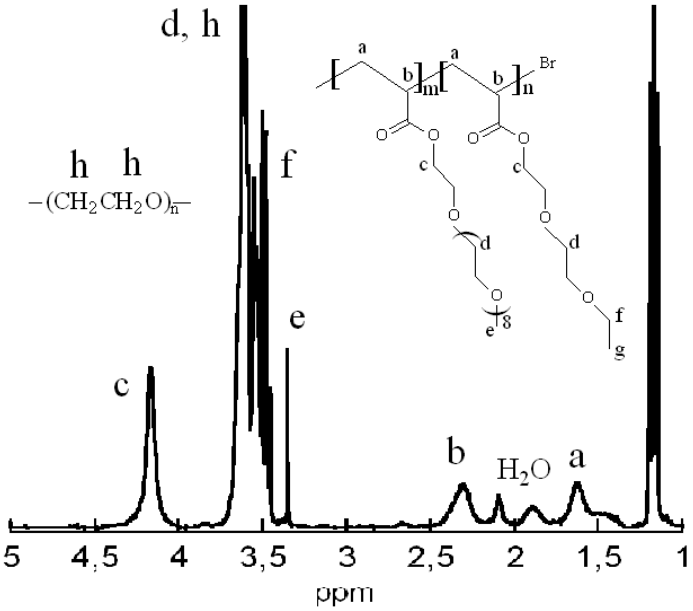


Fig. 2. ^{1}H-NMR spectrum of the $[P(DEGA\text{-}co\text{-}OEGA)]_2\text{-}PEG_{4000}\text{-}[P(DEGA\text{-}co\text{-}OEGA)]_2$ block copolymer (sample TO4-50)

The molecular weights and polydispersity of the synthesized block copolymers were determined by GPC in DMF, using a calibration curve obtained with polystyrene standards (Table 2, Fig. 3). Although the calibration with polystyrene standards does not indicate the real molecular weights, one can see that the measured MW follows the same increasing tendency as that expected, determined by the molecular weight of the PEG middle block and the target polymerization degree of the side blocks represented by the statistical copolymer (DEGA-OEGA) (Table 2). The polydispersity indices were in all cases larger ($M_w/M_n > 2$) than for a controlled process (Table 2), and the molecular weight

distributions were bimodal for the TO2-10, TO4-50 and TO6-50 samples and polymodal at higher molecular weights of the side blocks (TO4-75, TO4-100, Fig. 3). The block copolymer distribution displayed a shoulder at higher molecular weights. This suggests a termination process of the growing chains occurring mainly by radical combination, which could be a consequence of a slow deactivation of the growing chains by the Cu (II) complex, being more probable than the transfer reaction with the oxyethylene chains. All the attempts to improve the control of the polymerization reaction by adding $CuBr_2$, changing the solvent (ethanol, DMF, DMSO) or changing the catalytic system (CuBr/pentamethylethylenetriamine, CuBr/hexamethyltriethylenetetramine, CuCl/Me_6TREN, CuBr/Me_6TREN) have not led to a decrease of the polydispersity index.

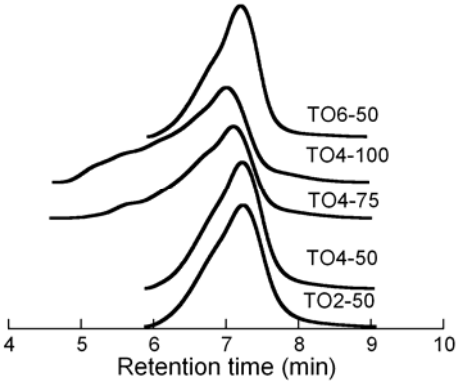


Fig. 3. GPC traces of the block copolymers obtained

Table 2

Molecular characteristics of the block copolymers synthesized and thermal gelation properties of their 20 wt% aqueous solutions

Sample	$M_{n,DHPEG}$ Da	Composition[a] (mol %)		M_n[b] g/mol	PDI[b]	T_{assoc}[c]	T_{gel}[d]	T_{syn}[d]
		DEGA	OEGA					
TO2-50	2060	94,2	5,8	21020	2,40	31	41	43
TO4-50	4010	94,1	5,9	22760	2,26	29	37	-[e]
TO4-75	4010	94,7	5,3	30550	5,62	25	28	-[e]
TO4-100	4010	94,4	5,6	39870	8,82	24	27	41
TO6-50	5850	94,2	5,8	26060	2,04	24	31[f]	44

[a] determined by ^{1}H-NMR (initial mole ratio DEGA/OEGA = 95/5) ; [b] determined by GPC ; [c] determined by rheometry on 20 wt% aqueous solutions ; [d] determined by the tube inversion method on 20 wt% aqueous solutions ; [e] syneresis did not occur up to 70ºC ; [f] the gel started to flow at 37ºC, but the syneresis did not occur up to 44ºC.

3.3. Thermogelation process

The thermal gelation properties of the block copolymers synthesized as a function of their structure were investigated on their 20 wt% aqueous solutions. Table 2 displays the results obtained by dynamic rheometry measurements and the tube inversion method.

The association temperature (T_{assoc}) was determined from dynamic rheometry measurements, carried out in "controlled deformation" mode, at a frequency of 1 rad/s and a heating rate of 1°C/min. T_{assoc} corresponds to the temperature at which the viscosity of the polymer solution starts increasing, and it characterizes the initial stage of the gelation process, i.e. the moment when the P(OEGA-co-DEGA) blocks start associating. All the polymers synthesized showed increased viscosity when the temperature was raised above a certain value (thermothickening behavior in aqueous medium), which can be explained by the formation of associations among the thermosensitive blocks as a consequence of the hydrophobic intermolecular interactions.

The measurements revealed that the temperature at which the solution viscosity started to increase depended on both molecular weight of the side arms and middle PEG block length. Thus, for the same length of the PEG middle block (TO4-50, TO4-75, TO4-100, Table 2, Fig. 4A) T_{assoc} decreased with increasing molecular weights of the copolymer P(OEGA-co-DEGA). This effect can be explained by the decrease of the polymer-solvent interactions as the molecular weight of side block increased. For similar molecular weights of the P(OEGA-co-DEGA) side blocks (TO2-50, TO4-50, TO6-50, Table 2, Fig. 4B), T_{assoc} decreased as the length of the PEG chain increased. This fact can be explained by the partial mixing of the P(OEGA-co-DEGA) and PEG chains which occurs during the association/aggregation process. The block copolymers synthesized represent a complex system due to the similar structure of the PEG middle chain and the side arms of oligo(ethylene glycol) acrylate units. The increase of the molecular weight of the PEG blocks determines the increase of the hydrophilic character of the block copolymer, as well as the increase of the segregation between the middle block and the side arms [25]. The former effect should lead to an increase of T_{assoc}, while the second one should produce a reduction of the hydrophilic influence of the PEG block onto the polyacrylate block, leading to a lower T_{assoc}, closer to the association temperature of the isolated polyacrylate block. In this case, the results showed that the predominant effect was the segregation.

A similar dependence on the molecular weights of both PEG and random copolymer was observed for the gelation temperature, determined by the inversion tube method for 20 wt% aqueous solutions. The results obtained indicate that the temperature at which the solution gelation occurs (i.e. the solutions do not flow

anymore when inverting the tube) depended on both polymerization degree of the thermoreversible block and PEG middle chain length, as in the case of T_{assoc}.

The tube inversion tests gave also the temperature at which syneresis occurred (i.e. water expulsion from the already formed gel). This is a common phenomenon for the thermoreversible hydrogels. Stable gels were obtained in the case of the block copolymers with PEG molecular weight of 4 kDa and polymerization degree of 50 and 75, respectively (TO4-50 and TO4-75 samples), when syneresis did not occur up to 70ºC. In all the other cases, the formed gels expelled water at temperatures slightly higher than 40ºC. An interesting behavior was displayed by sample TO6-50 as: i) it became a gel at 31ºC; ii) it started to flow while still remaining a very viscous liquid with a milky aspect at 37ºC; iii) it formed syneresis (a gel phase and a slightly viscous aqueous phase) at 44ºC.

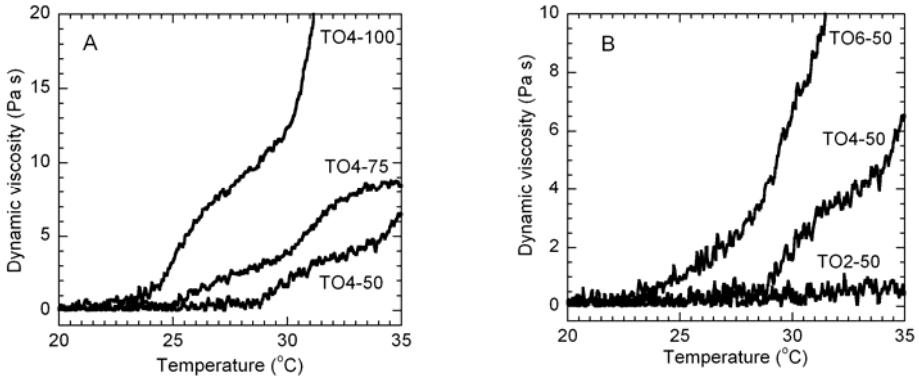


Fig. 4. Dependence of dynamic viscosity on the polymerization degree of the P(DEGA-co-OEGA) block (A) and on the PEG length (B)

The gels formed at 37°C from 20 wt% block copolymers aqueous solutions were also rheologically characterized by means of a rheometer working in oscillation mode, by using a parallel plate geometry. The amplitude sweep tests, carried out in controlled deformation mode at 1 rad/s (Fig. 5), confirmed the results obtained by the inversion tube method (Fig. 5A). Thus, the small plateau values of G' in the case of the TO2-50 (0.3 Pa) was due to the fact that the gel was not formed yet (T_{gel}<37°C), while for the TO6-50 (1.5 Pa) sample was due to the proximity of the syneresis temperature. The same proximity of the syneresis temperature determined a G' plateau value in the case of TO4-100 (40 Pa) located between those of TO4-50 (11 Pa) and TO4-75 (50 Pa), although, due to the higher molecular weight of the polyacrylates side arms, it should have been higher. Probably for the same reason, in the case of TO4-100, G' was lower than G", indicating a predominant viscous character. Unlike these, the samples TO4-50 and TO4-75, for which G' was higher than G" as far as the plateau values are concerned, showed a predominant gel-like behavior (Fig. 5B). It is worth noting

that, despite their behavior characteristic to a real gel (G'>G"), TO4-50 and TO4-75 are soft gels, G' value being much lower than 100 Pa.

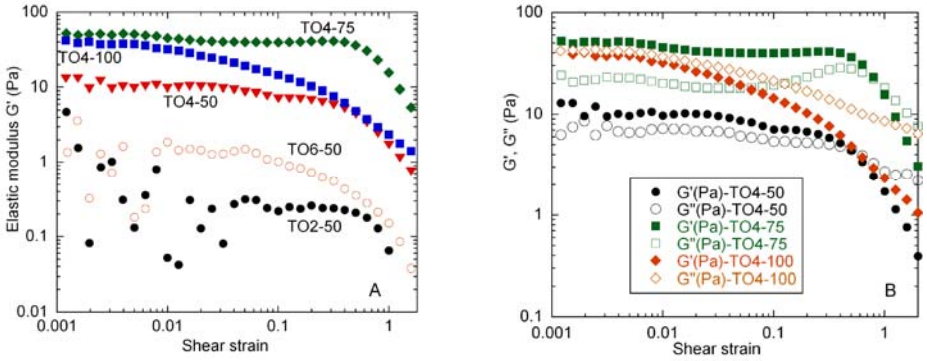


Fig. 5. Amplitude sweep tests. Tests conditions/parameters : 20 wt% aqueous polymer solutions, parallel plate geometry, plate diameter – 20 mm; gap – 0,5 mm; frequency-1 rad/s; controlled deformation mode ; 37°C

The frequency sweep tests (Fig.6), carried out within the linear viscoelastic region, showed that both G' and G" increased with frequency for TO4-50 and TO4-75. At low frequencies, including at rest, the 20 wt% solutions displayed a gel-like character at 37°C, the elastic modulus G' being larger than the viscous modulus G". However, as frequency increased, G" increased more than G', exceeding it at frequency values higher than the crossover frequency, when the solutions displayed a predominant viscous character. This behavior, which differs of that of a covalently crosslinked material (i. e. G'>G" for the entire frequency domain) may be explained by the reversible character and reduced strength of the forces involved in the formation of the analyzed hydrogels.

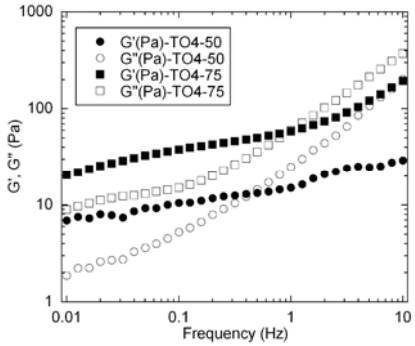


Fig. 6. Frequency sweep tests. Test conditions/parameters : 20 wt% aqueous polymer solutions, parallel plate geometry, plate diameter – 20 mm; gap – 0,5 mm; shear strain – 3%; controlled deformation mode ; 37°C

3.4. "In vitro" release of 5-FU

The synthesized block copolymers displaying a T_{gel} in the physiological range were studied as possible controlled drug release systems. The selected drug was 5-FU, which is one of the most used drugs in the gastrointestinal, respiratory etc. cancer treatment [26,27]. The release medium was a phosphate buffer solution (pH = 7,4), which simulates the physiological conditions, and also may reduce the phase separation temperature, due to its salts content (the so called "salting-out" effect). The experimental results (Fig. 7) indicated a slow drug diffusion from the formed hydrogel during more than a day. No relevant difference between hydrogels of various block lengths as far as the drug release rate is concerned was noticed.

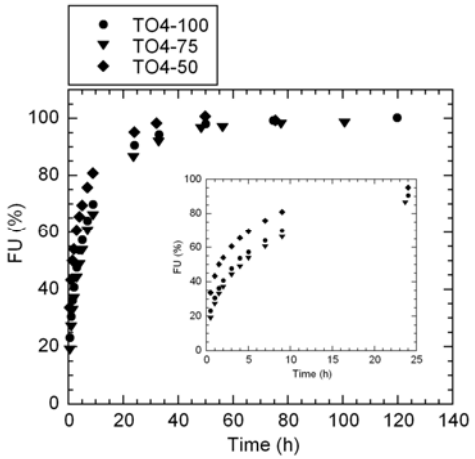


Fig. 7. Cumulative 5-FU release as a function of the time

Fig. 7 shows an initial drug release of about 20-30% of the initial loading amount within the first 30 min. This behavior is the so called "initial burst release", and it is often encountered for both chemical and physical hydrogels [27,28]. Then a sustained release (30-90% of the initial loading amount) was observed within 24 h, followed by an equilibrium stage, reached after 30 h only.

4. Conclusions

Novel branched block copolymers were synthesized by ATRP with a middle PEG chain and side arms represented by a random copolymer of di(ethylene glycol) ethyl ether acrylate with olig(ethylene glycol) methyl ether acrylate. The polymer synthesis was initiated by PEG tetrafunctional macroinitiators with around four 2-bromopropionate end groups, by using CuBr/bipy as catalytic system and toluene as solvent. The polydispersity indices

obtained were higher than 2, and the molecular weights distribution was bimodal in some cases (TO2-10, TO4-50 and TO6-50) and polymodal for higher molecular weights of the side blocks (TO4-75, TO4-100), indicative for termination reactions which occur during the polymerization process.

The synthesized block copolymers displayed a thermosensitive behavior in aqueous solutions, studied by both dynamic rheometry and the inverting tube method. The association temperature, as well as the gelation temperature depended on the molecular weight of the polymer blocks. The results showed that both T_{assoc} and T_{gel} decreased with the increasing of the PEG chain length. For the same molecular weight of the PEG middle block (4 kDa), T_{assoc} and T_{gel} depended on the polymerization degree of the acrylate copolymers, being lower at higher molecular weights of the side arms. The results obtained by the inverting tube method were confirmed by amplitude sweep tests. The frequency sweep tests carried out on the 20 wt% solutions which formed gel at 37°C, showed a gel-like behavior, i. e. G'>G", at lower frequencies. As frequency increased, both elastic and viscous modulus increased as well, and above a certain frequency value, the solutions presented a predominantly viscous character (G''>G'). This behavior was explained by the reversible character and reduced strength of the forces involved in the formation of the analyzed hydrogels.

The "in vitro" release tests of 5-fluorouracil proved the ability of these block copolymers to form injectable systems with slow drug release rate.

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