

Iranian Polymer Journal **17** (2), 2008, 125-133 Available online at: http://journal.ippi.ac.ir

Synthesis and Characterization of Biodegradable In Situ Forming Hydrogels via Direct Polycondensation of Poly(ethylene glycol) and Fumaric Acid

Azadeh Sadat Hashemi Doulabi¹, Shahriar Sharifi², Mohammad Imani^{2*} and Hamid Mirzadeh^{2,3}

(1) Biomedical Engineering Dept., Amirkabir University of Technology P.O. Box: 15875/4413, Tehran, Iran

(2) Iran Polymer and Petrochemical Institute, P.O. Box: 14965/115, Tehran, Iran

(3) Polymer Engineering Department, Amirkabir University of Technology P.O. Box: 15875/4413, Tehran, Iran

Received 22 September 2007; accepted 28 January 2008

A B S T R A C T

urrently, in situ forming polymeric gels and networks have attracted much attention due to their potential applications as injectable devices in drug delivery and tissue engineering. To this end, biodegradable hydrogels were developed based on poly (ethylene glycol)-co-fumaric acid macromers. These macromers were synthesized through direct polycondensation of poly (ethylene glycol) of different molecular weights and fumaric acid using dicyclohexylcarbodiimide (DCC) as an esterification promoting agent. They were fully characterized by spectroscopic methods (i.e., Fourier transform infrared and proton nuclear magnetic resonance spectroscopies) and gel permeation chromatography and thermal analysis (i.e., differential scanning calorimetry) techniques. Thermal characterization showed that the produced macromers have lower crystallinity than the original precursors. The molecular weight and the degree of oligomerization of the macromers were also substantially affected by the molecular weights of the initial PEGs. The macromers were cross-linked by redox mechanism in the presence of water soluble initiation system consisting of ammonium persulphate and N,N,N',N'-tetramethylethylenediamine. The swelling properties of the cross-linked gels were also examined which showed a significant dependency to the initial molecular weight of PEGs. Our results have suggested that these biodegradable hydrogels are potentially applicable in drug delivery and tissue engineering applications.

Key Words:

polyethylene glycol; unsaturated polyesters; direct polycondensation; in situ forming; hydrogels.

INTRODUCTION

During the last decade, an increasing number of in situ forming systems have been reported for a variety of medical applications such as drug delivery and tissue engineering [1-3]. Injection of a polymer/ cell or a polymer/drug mixture into the body can be regarded as the simplest and most convenient approaches in this way [2]. The advantages of these approaches are numerous. First, the initial materials are often liquids or mouldable putties thus, the devices are easily formed in complex shapes and are subsequently used to produce solid implants of the exactly required dimensions [3-5]. Secondly, the

^(*) To whom correspondence to be addressed. E-mail: M.Imani@ippi.ac.ir

invasive insertion and the removal process of traditional implants are reduced by administration of these materials via injection through customary needles. Finally, intimate contact with the surrounding tissues during the implant formation process will result to an improved adhesion [3,6].

Among a couple of possible strategies to achieve an in situ hydrogel formation system, using unsaturated polyesters seems as the suitable alternative candidates due to their potential ability to form cross-linked networks via their unsaturated double bonds [3-5,7]. Then, by using a photo-curing unit or any other safe chemical method the injected materials can be easily cured.

Fumaric acid containing macromers are highly unsaturated and can be cross-linked with or without using a cross-linking agent to form their corresponding polymeric networks [8]. Currently, a number of cross-linking agents are being used in these systems because they can enhance the polymerization efficiency while imparting specific properties to the network [9]. We have developed some novel unsaturated block copolymers based on fumaric acid and biodegradable polyesters, e.g., poly(caprolactone) and poly(hexamethylene carbonate) blocks in our laboratory during the last few years. The resulting materials have been used as drug delivery matrices to deliver anti-neoplastic agents such as tamoxifen citrate in breast cancer treatment or in situ forming biomedical devices, e.g., bone grafts or cements which were reported elsewhere [10-12].

The goal of this study was to prepare in situ forming hydrogels based on poly(ethylene glycol)-cofumarate macromers with possible drug delivery applications. Using acyl chlorides (fumaryl- or itaconvl chloride) to react with a diol has been the most frequently used synthetic pathway in our previous work [8] however, fumaric acid is much lower in terms of price in comparison with its corresponding acyl halide derivatives. Therefore, direct polycondensation of PEG and fumaric acid using dicyclohexylcarbodiimide (DCC) as an esterification-promoting agent has been accomplished and it is reported here to develop an economically more feasible material compared to previously synthesized PEGF hydrogels [9]. The resulting macromers have been cross-linked using water soluble redox initiation system comprising of ammonium persulphate/N,N,N',N'-tetramethylethylenediamine (APS/TEMED) as an effective method of generating free-radicals under mild aqueous condition [13] and the resulting hydrogel networks are well characterized.

EXPERIMENTAL

Materials

Poly(ethylene glycol)s (PEGs) (M_n s of 1 and 4 kDa) were purchased from Merck (Germany) and were dried by azeotropic distillation in toluene under reduced pressure, prior to use [3]. Ammonium persulphate, tetramethylethylenediamine (TEMED) were obtained from Fluka, Germany. Dicyclohexyl carbodiimide (DCC) and dimethylamino pyridine (DMAP) were received from Merck, Germany and used without any further purification. Fumaric acid, *N*-vinyl pyrrolidone, anhydrous dimethyl formamide (DMF) were all of analytical grade and purchased from Aldrich, USA. Other solvents were reagent grades and used without any further purification.

Synthesis

Poly(ethylene glycol)-*co*-fumarate (PEGF) macromers were prepared via direct polycondensation of PEG of different nominal number average molecular weights, i.e., 1 and 4 kDa with FA as represented in Scheme I. Typically, the dried PEG (0.025 mol) and fumaric acid (0.0225 mol) were dissolved in 200 mL of anhydrous DMF in a three-necked 500 mL glass reactor thermostated at 50°C in an oil bath. DCC (0.0225 mol) and DMAP (0.05% w/w of DCC) were also dissolved in DMF and added dropwise to the reaction flask over 1 h while the reaction mixture was vigorously stirred. The reaction was run overnight at 50°C.

Upon completion of the reaction, the mixture was cooled down and dicyclohexyl urea (DHU) precipitated and the solvent was removed by filtration and rotovaporation, respectively. The residue was then dissolved in 200 mL of warm 1,2 propane diol and then recrystallized twice. In order to avoid premature cross-linking at room temperature, the resulting unsaturated polyester was kept in a refrigerator at 5°C [14,15].



Scheme I. The synthetic steps of PEGF.

Characterizations

Fourier transform infrared (FTIR) spectra (4000-400 cm⁻¹) were obtained on a Bruker, Equinox 55 spectrophotometer at 4 cm⁻¹ resolution and 32 scans. All samples were prepared as KBr discs. The macromers were also characterized by proton nuclear magnetic resonance (¹H NMR) spectroscopy at 400 MHz and 8 scans on a Bruker 400 ultrashield[™]. CDCl₃ was used as solvent and chemical shifts were given in ppm. Melting point (T_m), heat of fusion, and crystallinity (χ) were obtained via a TA 920 (Germany) differential scanning calorimeter (DSC) under nitrogen gas flow rate of 50 mL/min and at a heating rate of 10°C/min from -100°C to 100°C. The percent crystallinity of PEGFs and their corresponding initial PEGs, (χ) , were determined using the following equation [8]:

$$\chi = \frac{\Delta H_{\rm m}}{\Delta H_{\rm m}^*} \times 100 \tag{1}$$

where, ΔH_{m}^{*} is the theoretical heat of fusion of 100% crystalline PEG (49 cal/g) [16]. The molecular weight (macromers and the corresponding PEG precursors) were determined using gel permeation chromatography (GPC) technique on a CR4AX GC15A Shimadzu (Japan) instrument equipped with a refractive index detector. Polystyrenes of known molecular weights were used as the calibration standards and reagent grade THF was used as the mobile phase eluting at flow rate of 1.0 mL/min. A 100 µL sample of 0.1 mg/mL solution of polymer dissolved in THF (which was filtered through a 0.22 µm filter prior to use) was injected for each measurement.

Cross-linking of Unsaturated Polyesters

Unsaturated polyesters were cross-linked by redox polymerization in the presence of chemical initiators. Ammonium persulphate (250 mM) and TEMED (250 mM) as initiator system [17] and an optimized

Iranian Polymer Journal / Volume 17 Number 2 (2008) 127



Scheme II. Cross-linking of PEGF.

amount of NVP as a cross-linking agent were added to the mixture and mixed thoroughly. The concentration of the macromer was kept constant (33 wt%) at all formulations. Swelling ratio and sol fraction percentage were studied in water. To find the effects of redox initator system concentration on the network characteristics, the macromer was cross-linked in the presence of APS/TEMED in different concentrations of 50, 150, and 250 mM. The cross-linked samples were dried in a convection oven at 25°C for 24 h and stored in a desiccator at 4°C until use. Schematic representation of the reaction is shown in Scheme II.

Equilibrium Swelling Studies

Statistically significant number of samples comprising the cross-linked macromers (prepared in different conditions) were weighed (W_i) and put in 20 mL of deionized distilled water until an equilibrium was reached. Then, they were weighed (W_s) again after blotting the superficial water. The swollen gels were dried at reduced pressure for 24 h at 25°C and weighed again (W_d). The equilibrium weightswelling ratio was obtained when the weight of the swollen hydrogel reached a constant value. Swelling ratio (SR) and sol fraction percentage were determined from the following equations [4]:

Swelling ratio =
$$\frac{W_s - W_d}{W_d}$$
 (2)

Sol fraction (%) =
$$\frac{W_i - W_d}{W_i} \times 100$$
 (3)

Rheology Study

PEGFs 1 kDa or 4 kDa and the corresponding amount of the cross-linker, i.e., NVP were dissolved in the initiator combination APS/TEMED (250 mM) solution. The concentration of the macromer was kept constant (33 wt%) in all formulations. The complex viscosity (η^*) of these solutions was monitored via a dynamic oscillatory test at 37°C with a rheometer (MCR300, Paar Physica, Germany) in parallel plate geometry.

Briefly, the polymer solution was placed in the stainless steel cup (9 mm in diameter and 20 mm in height) thus, it filled two third of the cup height. The upper plate (8 mm diameter) was lowered until it contacted with the surface of the solution and the rheological behaviour was monitored at amplitude gamma = 1 % and angular frequency (ω)=10 rad/s. Gelation onset was determined to be the first time at which there were at least 4 consecutive points with 20% change in complex viscosity. Gelation completion was considered the first time at which there were at least 4 consecutive points with 20% change in the η^* [18].

RESULTS AND DISCUSSION

Figure 1 shows the FTIR spectra of PEG precursors and PEGFs 1 and 4 kDa. On the spectra of macromers the characteristic ester carbonyl stretching bond at 1721 cm⁻¹, asymmetrical C-O-C stretching bond at 1110 cm⁻¹, C-H stretching bond at 2869 cm⁻¹, C=C stretching bond at 1644 cm⁻¹, methylene scissoring and asymmetric peaks at 1454 cm⁻¹ were detected. According to the FTIR spectra, the macromers showed mostly terminal fumarate carboxyl functional groups which is evident by the weakening of the broad -OH end groups absorption at 3500 cm⁻¹.

The ¹H NMR spectra of PEG 1 kDa, PEGF 1 kDa, and PEGF 4 kDa in Figure 2 show four major signals



Figure 1. FTIR Spectra of PEG 1 kDa, PEGF 1 kDa, and PEGF 4 kDa.

for all the macromers. The peak at 3.63 ppm is attributed to the methylene protons on the PEG backbone. Deshielded protons of fumarate units were positioned at 6.88 ppm. Due to chemical environment and neighbouring effects, signals of PEG methylens adjacent to the fumarate functional groups were shifted to



Figure 2. ¹H NMR Spectra of PEG 1 kDa, PEGF 1, and PEGF 4 kDa.

downfield, i.e., 4.33 ppm. As a common feature, the OH proton signal position which is sensitive to temperature and concentration appeares at 2.7 ppm.

According to the supposed reaction mechanism on the production of the ester bonds in presence of DCC and DMAP [19] the fumaric acid is converted by DCC to its corresponding anhydride species (Scheme I) which forms an acyl pyridinium species with DMAP (1). This is followed by equilibration (1) with the PEG diol to produce ion pair (2). The nucleophilic attack by R'O- on the acyl group of (2) has generated the ester and DMAP is also recovered.

On the other hand, the reaction mechanism can be explained by the formation of a reactive Oacylisourea intermediate by DCC and fumaric acid which offers reactivity similar to the corresponding fumaric acid anhydride (maleic anhydride). At this stage poly(ethylene glycol) (PEG), is added to the activated fumaric acid to form stable dicyclohexylurea (DHU) and the ester.

Direct polycondensation reaction may be accomplished using only diol and diacid in presence of Lewis acids or carried out by azeotropic distillation in presence of protic acids. However, in such cases high temperature is required and finally low yields are obtained [20]. Using DCC and DMAP (5 mol% relative to DCC) pair enhanced the reaction as previously described in the literature. In practice, the reaction of fumaric acid, DCC, and amines easily lead to the formation of the corresponding amides without any problem [21].

Melting point (T_m) , crystallinity (χ) and heat of fusion (ΔH_m) obtained from DSC and molecular weights from GPC experiments are summarized in Table 1. Melting point and heat of fusion of PEGFs were lower than their corresponding precursors which can be attributed to the changes in the chains

Table 1. Summery characteristics of PEGs and PEGFs.

Sample	T _m (°C)	∆H _m (mcal/mg)	χ (%)	M _n (g/mol)	M _n (g/mol)
PEG 1 kDa	47.77	21.10	43.06	955	1080
PEGF 1 kDa	46.10	17.67	36.06	2470	4670
PEG 4 kDa	68.49	25.37	51.77	5100	6000
PEGF 4 kDa	54.80	22.31	45.53	6066	11404

Iranian Polymer Journal / Volume 17 Number 2 (2008)

Table 2. Values of sol fractions and swelling ratios for different PEGFs in 10 μ L Nrpsanples (n=3).

APS (TEMED) conc.		Sol fraction	Swelling
(mM)		(%)	ratio
PEFG 1 kDa	50	34.1±0.43	4.2±0.62
	150	17.4±1.10	6.3±1.02
	250	8.1±1.01	10.1±0.96
PEFG 4 kDa	50	49.2±1.01	9.6±0.31
	150	23.3±1.02	13.1±0.08
	250	12.5±0.3	16.0±1.27

conformation upon introduction of a rigid doublebond in the backbone. Decreasing chain flexibility by incorporating these rigid fumarate bonds prevent close packing of flexible PEG chains. Thus, a decrease in crystallinity and T_m is observed as is shown in Table 1. Then again, there could be an increased tendency in higher molecular weight PEGFs and their PEG precursors to have more crystalline characteristics in comparison with those of lower molecular weights. It is owing to the higher segmental mobility provided by higher molecular weights of the initial PEG precursors. This can provide more thermodynamically possible conformations which in turn increase close packing and hence the crystallization potential [8]. This increased crystallinity may result in the observed changes in T_m and heat of fusion in comparison with their lower molecular weight analogs.

In order to optimize the PEGF cross-linking reaction, formulations were prepared with varying concentrations of initiator/accelerator (1:1 molar ratio) and sol fraction and swelling ratios were then determined as are cited in Table 2. The amount of NVP in the formulations was also optimized via adding an increasing amount (5, 8, 12, 20, and 80 %w/w) of NVP to the macromers and obtaining sol fraction, swelling ratio, and shrinkage strain rate results. The ratio of 12% (10 μ L) was found as an optimum value for NVP concentration in both 1 kDa and 4 kDa macromers.

The lowest amounts of sol fraction were found for the samples containing 250 mM of initiator/accelerator system hence, the rheometrical studies were performed just on these samples. High sol fraction values



Figure 3. UV spectra of sol fraction supernatant.

are a common phenomenon in the hydrogels [8,22]. Different values for sol fraction were obtained for different formulations in the range of 8-49%. Upon optimization of APS and TEMED concentrations, i.e., 250 mM the least values were obtained in the range of 10%. High sol fraction values cannot be regarded as a biocompatibility problem per se and as we found no such problem with the optimized formulations based on in vitro biological tests reported before [5]. It may be upon this fact that NVP will not be the only constituent of the sol fraction due to consumption of NVP as a cross-linking agent in the reaction with fumarate double bonds. In fact NVP is converted to PVP oligomers which are evidently biocompatible [23, 24]. The remaining parts of the sol fraction consist of biocompatible PEGs and PEGFs. UV spectra (UV-1650 PC, Shimadzu, Japon) of sol fraction supernatant were also depicted in Figure 3 which clearly does not show any trace of NVP (Figure 3).

As seen in Table 2 the swelling ratios were



Figure 4. Complex viscosity vs. time profiles of PEGFs 1 and 4 kDa at 37°C.

increased significantly upon an increase in the molecular weight of the corresponding PEG precursors. Higher degree of oligomerization of PEGF 1 kDa (in comparison with PEGF 4 kDa provide lower molecular weight between cross-links for this macromer. This phenomena in conjunction with lower chain length in PEGF 1 kDa macromers may result in a denser network and a less dense for PEGF 4 kDa (in comparison) which provides higher swelling ratios for the networks made of higher molecular weight macromers and hence of initial PEGs. The variation of some properties like molecular weight between the cross-links and mesh size between the cross-linkable fumarate bonds, which are both affected by PEG molecular weight and initiator/accelerator system concentration may change swelling ratio which is currently under investigation.

The components of redox initiation system have shown a significant effect on the sol fraction (Table 2). The increase in APS concentration has resulted in a decrease in the sol fraction and increase in the swelling ratio, which could be due to an increased free-radical production [13]. Sol fraction of the hydrogels synthesized via DCC method is higher than what previously reported [8] using triethylamine (TEA) or potassium carbonate (K_2CO_3) as proton scavengers in PEGF synthesis. This can be attributed to the lower conversion in the esterification reaction and less fumarate double bond in the backbone that can be cross-linked by using NVP [8,16].

Typical data generated by monitoring the complex

(APS/TEMED concentration=250 mM), n=3.				
Sample	Onset (min) ± SD	Completion (min) ± SD		

Table 3. Times to onset and completion of gelation,

Sample	Onset (min) ± SD	Completion (min) ± SD	
PEGF 1 kDa	3.5 ± 0.6	51.6 ± 7.5	
PEGF 4 kDa	3.0 ± 0.5	28.0 ± 5.9	

viscosity over time during the cross-linking reaction are shown in Figure 4. Detailed results on the onset and completion of the gelation reaction for PEGFs 1 kDa and 4 kDa samples containing optimum amounts of NVP and APS/TEMED have appeared in Table 3. The onset times for PEGFs 1 kDa and 4 kDa were determined by rheometry to be 3.5 and 3 min, respectively. Statistical analysis (by paired t-test) with p<0.05 have revealed shorter onset and completion of gelation times for PEGF 4 kDa than the PEGF 1 kDa formulation, which is probably due to the former tendency for rapid aggregation with increasing of its molecular weight. In contrast to the hydrogels synthesized via acylation method catalyzed by TEA, the onset time of gelation was almost identical, although the completion time of gelation decreased by increasing the molecular weight of the macromers [25].

CONCLUSION

The unsaturated polyester based on PEG and fumaric acid was prepared via direct polycondensation reaction using DCC and DMAP to remove the produced water due to the esterification reaction and also catalyze and promote the reaction. The ¹H NMR and FTIR spectroscopic results clearly indicated that the fumarate groups have been incorporated into the macromer backbone. According to the results T_m, ΔH_m , M_w , M_n , χ , swelling ratio, and sol fraction were impressed by PEG molecular weight. Higher increase in M_w results in more increase in the crystallinity of the macromers. Due to the unsaturated fumarate bonds these macromers are potentially cross-linkable. The cross-linking reaction carried out using redox initiation system has resulted in PEGF gels exhibiting typical properties of the hydrogels depending on the molecular weight of PEG precursor and initiator concentration. The PEGF 4 kDa had shorter onset time and also completion of gelation in a significantly shorter time than the PEGF 1 kDa. Application of these biodegradable copolymers for delivery of antineoplastic agents is currently under investigation.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Iran National Scientific Foundation (INSF grant # 8314) and Iran Polymer and Petrochemical Institute (IPPI grant # 23741109) for their kind financial support.

REFERENCES

- Chen T, Embree HD, Brown EM, Taylor MM, Payne GF, Enzyme-catalyzed gel formation of gelatin and chitosan: Potential for in situ applications, *Biomaterials*, 24, 2831-2841, 2003.
- Balakrishnan B, Jayakrishnan A, Self-crosslinking biopolymers as injectable in situ forming biodegradable scaffolds, *Biomaterials*, 26, 3941-3951, 2005.
- Shu XZ, Liu Y, Plumbo FS, Luo Y, Prestwich GD, In situ crosslinkable hyaluronan hydrogels for tissue engineering, *Biomaterials*, 25, 1339-1348, 2004.
- 4. Sanborn TJ, Messersmith PB, Barron AE, In situ crosslinking of a biomimetic peptide-PEG hydrogel via thermally triggered activation of factor XII, *Biomaterials*, **23**, 2703-2710, 2002.
- Temenoff JS, Shin H, Conway DE, Engel PS, Mikos AG, In vitro cytotoxicity of redox radical initiators for crosslinking of oligo(poly(ethylene glycol) fumarate) macromers, *Biomacromolecules*, 4, 1605-1613, 2003.
- Pachaeuser CB, Schnieders J, Oster CG, Kissel T, In situ forming parenteral drug delivery systems: An overview, *Eur J Pharm Biopharm*, 58, 445-455, 2003.
- Ruel GE, Leroux JC, In situ hydrogels: Review of temperature-sensitive systems, *Eur J Pharm Biopharm*, 58, 409-426, 2003.
- Jo S, Shin H, Shung AK, Fisher JP, Mikos AG, Synthesis and characterization of oligo(poly(ethylene glycol) fumarate) macromer, *Macromolecules*,

34, 2839-44, 2001.

- Timmer MD, Jo S, Wang C, Ambross CG, Mikos AG, Characterization of the crosslinked structure of fumarate-based degradable polymer networks, *Macromolecules*, 35, 4373-4379, 2002.
- Hashemi AS, Sharifi S, Imani M, Mirzadeh H, Atai M, Biodegradable and in situ visible light cureable polyethylene glycol based hydrogels for tumor delivery of tamoxifen citrate, 34th Ann Meet Expo Control Rel Soci proc (CRS2007). Long Beach, California, 7th-11th July, 2007.
- Sharifi S, Imani M, Mirzadeh H, Atai M, Bakhshi R, Ziaee F, Synthesis and characterization of novel injectable, biodegradable and in situ crosslinkable poly(hexamethylenecarbonate-fumarate), poly (hexamethylene carbonateacry-late) and poly(ethylene glycol fumarate-co-hexamethylene-carbonate-fumarate) scaffolds for bone tissue engineering, 28th Ann Int Conf IEEE Eng in Med Bio Soc Proc, New York, 30th Aug- 3rd Sep, 2006.
- Sharifi S, Hashemi A, Imani M, Mirzadeh H, Atai M, Ziaee F, Synthesis of poly(ε-caprolactone fumarate) via acylation using a new proton scavenger as an injectable and in situ forming drug carrier, *7th Int Symp on Front of Biomed Polym* (*FBPS2007*), Ghent, Belgium, 24th-27th June, 2007.
- Behravesh E, Jo S, Zygourakis K, Mikos AG, Synthesis of in situ crosslinkable macroporous biodegradable poly(propylene fumarate-*co*-ethylene glycol) hydrogels, *Biomacromolecules*, 3, 374-381, 2003.
- Pielichowski K, Flejtuch K, Differential scanning calorimetry studies on poly(ethylene glycol) with different molecular weights for thermal energy storage materials, *Polym Adv Technol*, 13, 690-696, 2002.
- 15. Temenoff JS, Shin H, Conway DE, Engel PS, Mikos AG, In vitro cytotoxicity of redox radical initiators for cross-linking of oligo(poly(ethylene glycol)fumarate) macromers, *Biomacromolecules*, **4**, 1605-1613, 2003.
- 16.Wang S, Lu L, Gruetzmacher JA, Currier BL, Yaszemski MJ, Synthesis and characterizations of biodegradable and crosslinkable poly(ε-caprolactone fumarate), poly(ethylene glycol fumarate),

and their amphiphilic copolymer, *Biomaterials*, **27**, 832–841, 2006.

- 17.Park H, Temenoff JS, Tabata Y, Caplan A, Mikos AG, Injectable biodegradable hydrogel composites for rabbit marrow mesenchymal stem cell and growth factor delivery for cartilage tissue engineering, *Biomaterials*, 28, 3217-3227, 2007.
- Petrova T, Manolova N, Rashkov L, Li S, Vert M, Synthesis and characterization of poly(oxyethylene)-poly(caprolactone) multiblock copolymers, *Polym Int*, 45, 419-426, 1998.
- 19. Hassner A, Alexanian V, Direct room temperature esterification of carboxylic acids, *Tetra Lett*, **46**, 4475-4478, 1978.
- Heller J, Helwing RF, Controlled release of watersoluble macromolecules from bioerodible hydrogels, *J Cont release*, 4, 262-266, 1983.
- 21. Temenoff JS, Park H, Jabbari E, Conway DE, Sheffield TL, Ambrose CG, Mikos AG, Thermally crosslinked oligo(poly(ethylene glycol) fumarate) hydrogels support osteogenic differentiation of encapsulated marrow stromal cells in vitro, *Biomacromolecules*, **5**, 5-10, 2004.
- 22. Shin H, Jo S, Mikos AG, Modulation of marrow stromal osteoblast adhesion on biomimetic oligo[poly(ethylene glycol) fumarate] hydrogels modified with Arg-Gly-Asp peptides and a poly(ethylene glycol) spacer, *J Biomed Mater Res*, **61**, 169–179, 2002.
- Gresser JD, Hsu SH, Nagaoka H, Lynos CM, Analysis of a vinyl pyrrolidone/poly(propylene fumarate) resorbable bone cement, *J Biomed Mater Res*, 29, 1241-1247, 1995.
- 24.Higa OZ, Rogero SO, Machado LDB, Mathor MB, Lugão AB, Biocompatibility study for PVP wound dressing obtained in different conditions, *Radiat Phys Chem*, 55, 705-707, 1999.
- 25.Kim B, Peppas NA, Poly(ethylene glycol) containing hydrogels for oral protein delivery applications, *Biomed Microdevices*, **5**, 333-341, 2003.