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ORIGINAL ARTICLE

Synthesis of new poly(ether–urethane–urea)s based on amino acid cyclopeptide and PEG: study of their environmental degradation

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Abstract Conventional polyurethanes (PUs) are among biomaterials not intended to degrade but are susceptible to hydrolytic, oxidative and enzymatic degradation in vivo. Biodegradable PUs are typically prepared from polyester polyols, aliphatic diisocyanates and chain extenders. In this work we have developed a degradable monomer based on α-amino acid to accelerate hard segment degradation. Thus a new class of degradable poly(ether–urethane–urea)s (PEUUs) was synthesized via direct reaction of 4,4′-methylene-bis(4-phenylisocyanate) (MDI), L-leucine anhydride (LA) and polyethylene glycol with molecular weight of 1,000 (PEG-1000) as polyether soft segment. The resulting polymers are environmentally biodegradable and thermally stable. Decomposition temperatures for 5 % weight loss occurred above 300 °C by TGA in nitrogen atmospheres. Some structural characterization and physical properties of these polymers before and after degradation in soil, river water and sludge are reported. The environmental degradation of the polymer films was investigated by SEM, FTIR, TGA, DSC, GPC and XRD techniques. A significant rate of degradation occurred in PEUU samples under river water and sludge condition. The polymeric films were not toxic to *E. coli* (Gram negative), *Staphylococcus aureus* and *Micrococcus* (Gram positive) bacteria and showed good biofilm formation on polymer surface. Our results show that hard segment degraded selectively as much as soft segment and these polymers are susceptible to degradation in soil and water. Thus our study shows that new environment-friendly polyurethane, which can degrade in soil, river water and sludge, is synthesized.

Keywords Biodegradable segmented copolyurethane · PEG · Amino acid anhydride · Cell-toxicity

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Introduction

Polyurethanes (PUs) are a widely used class of polymer with excellent mechanical properties and good biocompatibility (Gorna and Gogolewski 2002a, b; NIIR Board 2006; Gunatillake et al. 2006; Tang et al. 2001; Hiltunen et al. 1998). They have been evaluated for variety of biomedical applications such as coating materials for breast implants, pacemaker leads, catheters and prosthetic valve leaflets (Tang et al. 2001; Gorna and Gogolewski 2002a, b; Santerre et al. 2005). For some of these applications such as vascular prostheses, artificial skin, pericardial patches, soft-tissue adhesive, drug delivery devices, scaffolds for tissue engineering, besides biocompatibility, biodegradability is a required factor (Nayak et al. 2001; Guan et al. 2005; Bruin et al. 1990; Warrer et al. 1992). Biodegradability of polyurethanes is generally achieved by incorporating labile and hydrolysable moieties into the polymer backbone. The most common method for fulfilling this goal is the application of polyols (soft segments) with hydrolysable bonds as starting materials for the preparation of polyurethanes. Several hydroxyl-terminated polymers such as polycarolactone, polyalkylene adipate, polylactides and poly glycolides were used for the synthesis of hydrolytically degradable polyurethanes (Yeganeh et al. 2005, 2006; Gorna and Gogolewski 2002b). In addition

poly(ether–urethane)s (PEU) show good biocompatibility, hydrolytic stability and good low-temperature properties. However; their degradation rate is to some extent slow. On the other hand, it was shown that copolymers of PEG and peptide sequences were designed in which PEG has properties appropriate for incorporation in polymers that are highly water-soluble, nonimmunogenic, degradable and relatively non-toxic (Matthews et al. 2000). Hydrolytic-microbial degradation of PEG has also been studied by some groups. It was shown that degradation of PEG occurred as a combination of oxidation and hydrolysis. Also partial wet air oxidation converted PEG-10000 into lower-molecular-weight compounds (Okada 2002). In addition the synthesis of polymers containing amino acids residue is a subject of much interest, since a high degree of amino acid functionality can lead to polymers with increased solubility and the ability to form secondary structures. Because the amino acids are naturally occurring compounds, synthetic polymers based on amino acids are anticipated to be nontoxic, biocompatible and biodegradable. On the other hand, synthetic polymers containing amino acid residues in the main chain or in the side chain can be employed for biomedical applications (Torma et al. 2007; Mallakpour and Zadehnazari 2011).

In order to combine aforementioned properties and increase degradation rate of PEUs, in this study a new class of (bio)degradable PEUs containing PEG and an amino acid-based monomer was prepared. Some structural characterization and physical properties of these polymers before and after degradation in environment were studied.

Experimental

Materials and methods

All chemicals were purchased from Fluka Chemical Co. (Buchs, Switzerland), Aldrich Chemical Co. (Milwaukee, WI), Riedel-deHaen AG (Seelze, Germany) and Merck Chemical Co. MDI (Aldrich, 98 %) was used as received. PEG-1000 was purchased from Merck and was dried under vacuum at 80 °C for 8 h. *N,N*-Dimethyl formamide (DMF), *N*-methyl-2-pyrrolidone (NMP) (Merck, 99.5 %) were distilled under reduced pressure over BaO (Aldrich, 97 %). Dibutyltin dilurate (DBTDL) (Merck, 97 %) and (S)-(+)-Leucine (Merck, 99 %) were used as received. Ethylene glycol (EG) (Merck, 99 %) was distilled under reduced pressure over CaO (Merck, 97 %).

FT-IR spectra were recorded on a Jasco FT-IR spectrophotometer. Spectra of solids were carried out using KBr pellets. Vibrational transition frequencies are reported in wave number (cm^{-1}). Band intensities are assigned as weak (w), medium (m), shoulder (sh), strong (s), broad (br), stretching (st.) and bending (bend). Proton nuclear magnetic resonance ^{1}H-NMR (400 MHz) spectra were recorded on a Bruker, Avance 400 instrument in dimethyl sulfoxide (DMSO-d_6) at room temperature (RT). Multipilicities of proton resonance were designated as singlet (s), doublet (d), doublet of doublet (dd), multiplet (m) and broad (br). Inherent viscosities were measured by a standard procedure using a Cannon-Fensk Routine Viscometer. Thermal Gravimetric Analysis (TGA) data for polymers were taken on a Mettler-Toledo TG-50 Thermal Analyzer under N_2 atmosphere at heating rate of 20 °C/min. The initial and peak temperatures were read at the beginning and in the middle of the decomposition step obtained by TGA curve. Differential Scanning Calorimetery (DSC) data for polymers were recorded on a DSC-30/S instrument under N_2 atmosphere. Glass transition temperatures (T_g) were read at the middle of the transition in the heat capacity taken from heating DSC traces. A sample was first scanned from room temperature to 110 °C and maintained for 1 min followed by quenching to −100 °C at a cooling rate of 10 °C/min, and then a second heating scan was used to measure sample's T_g of soft (T_{gs}) or hard segment (T_{gh}). A heating rate of 10 °C/min was applied to all samples. Scanning electron microscopy (SEM) study was performed with a field emission EM XL30 Philips Scanning Electron Microscope, after samples were metallized with gold. Wide-angle X-ray diffraction measurements (WAXS) were carried out with a Bruker, D8 advance XRD Diffractometer using a graphite monochromatized Cu Kα radiation (40 kV; 40 mA). The number (Mn) and weight average molecular weight (Mw) and polydispersity index (PDI) of the polymer samples were determined using a gel permeation chromatography system (Manager 5000-Knauer-GPC). DMF was used as eluent at a flow rate of 0.5 mL min^{-1} at RT. Monodispersed polystyrene standards were used to obtain a calibration curve.

E. coli, *Staphylococcus aureus* and *Micrococcus* bacteria grew in Petri dishes on Nutrient agar. Once the growth media in the Petri dishes were inoculated with the desired bacteria, the plates were incubated (usually at 37 °C) in the presence of small pieces of polymer films.

Nutrient agar-based growth medium typically contains a carbon source for bacterial growth, which may be a sugar such as glucose, or a less energy-rich source like succinate, various salts, which may vary among bacteria species and growing conditions; these generally provide essential elements such as magnesium, nitrogen, phosphorus and sulfur to allow the bacteria to synthesize protein and nucleic acid, water. The medium comprised a basal medium [0.2 % KH_2PO_4; 0.7 % K_2HPO_4; 0.1 % $(NH_4)_2SO_4$; 0.01 % $MgSO_4 7H_2O$; 0.0001 % $ZnSO_4 7H_2O$; 0.00001 % $CuSO_4 7H_2O$; 0.001 % $FeSO_4 7H_2O$; 0.0002 % $MnSO_4 H_2O$; the final pH was 7.2].

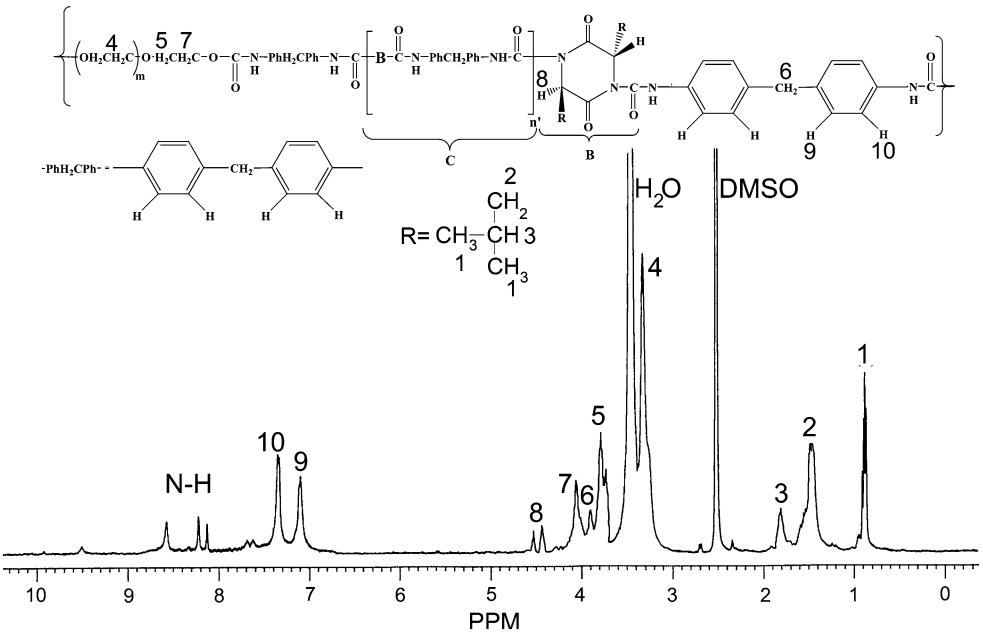


Fig. 1 ^{1}H NMR (400 MHz) spectrum of PEUU in DMSO-d_6 at RT

Polymer synthesis

The general procedure for preparation of PEUU by two-step pre-polymerization method is as follows: Into a dried 25 mL round bottom flask with an addition inlet, occupied with drying tube and N_2 balloon, LA (0.2 g, 8.67×10^{-4} mol) was dissolved in 0.15 mL of NMP 1 % (NMP containing 1 % w/w LiCl) at 110 °C; after decreasing temperature a solution of MDI (0.443 g, 17.34×10^{-4} mol) in 0.15 mL of NMP 1 % was added at 80 °C. The solution was heated at 80–90 °C for 2 h and at 120 °C for 2 h. During this period appropriate amounts of NMP 1 % were added. Then it was cooled to 50 °C, and a solution of PEG-1000 (0.884 g, 8.67×10^{-4} mol) in 0.3 mL of NMP 1 % was added. The reaction mixture was stirred between 60 and 70 °C for 1 h, then it was heated up to 110 °C over a period of 10 h, and NMP 1 % was also added. The total solid content of reaction mixture was kept at about 50 % W/V. Then the viscous solution of reaction mixture was poured into 5 mL of methanol–water as non-solvent. After vigorous grinding and stirring for 0.5 h, precipitated polymer was isolated. For additional purification, fractional precipitation method was applied by re-dissolving and re-precipitation of polymer in DMF (or DMF 1 %) and methanol–water, respectively. The precipitated polymer was collected by filtration and dried at 80 °C for 6 h under vacuum to give 1.115 g (73 % yield) of polymer. Inherent viscosity of the resulting copolymer was 0.35 dL/g. The FT-IR and ^{1}H-NMR spectra were consistent with the assigned structure. FT-IR Peaks (cm^{-1}): 3,314 (m, br) NH st., 3,301 (w, br) NH st., 3,090 (w), 3,046 (w) CH aromatic st., 2,955 (m) CH st., 1,772 (w, sh) C=O urethane st., 1,676 (s,) C=O urethane-amide st, 1,597 (m) C=C st., 1,557 (m) C–N st. + NH bend, 1,538 (s) C–C st., 1,510 (m) C=C st., 1,410 (m) C–N st., 1,400 (m) CH bend, 1,307 (m), 1,227 (m) C–N st. + NH bend, 1,203 (m), 1,199 (w), 1,105 (m) C–O–C ether st, 1,042 (m) O=C–O–C st., 1,018 (w), 917 (w), 850 (w), 814(w) NH bend, 756 (w) O=C–O, 663 (w), 510 (w), 482 (w). ^{1}H-NMR peaks, DMSO-d_6 at RT, δ ppm: 0.91 (d, CH_3(1), 6H), 1.5 (t, CH_2(2), 2H), 1.8 (m, CH(3), 1H), 3.4 (t, CH_2(4), 4H), 3.8 (t, CH_2(5), 2H), 3.9 (s, CH_2(6), 2H), 4.1 (t, CH_2(7), 2H), 4.5 (t, CH(8), 1H), 7.1 (CH(9), 4H), 7.4 (CH(10), 4H), 8.2 (br, NH), 8.25(br, NH), 8.6 (br, NH) (Fig. 1).

In vitro degradation tests

Film preparation

In order to study the environmental degradation of resulting polymers, PEUU films with 10 × 20 mm in size and approximately 0.2-mm thickness were cast from NMP solution of polymers into Teflon molds. The films were stored at RT for 1 week before use to reach the equilibrium crystallinity. Then the films were dried in vacuum at 50 °C prior to the experiments. Polymer mass was determined using a Mettler Toledo AB204-S Classic balance. Samples were stored in plastic bags in a desiccator prior to in vitro degradation tests. Then they were immersed or buried in selected environment using tea bag method (Fig. 2) to prevent tearing or fragmentation of the samples.

Polymer samples were stored in river water, soil and sludge at RT for more than 4 months. Every 15 days PEUU specimens were withdrawn from their container, placed in water to remove environmental impurities and then placed under nitrogen and subsequently vacuum at 50 °C for 24 h until a constant weight was reached. Post-

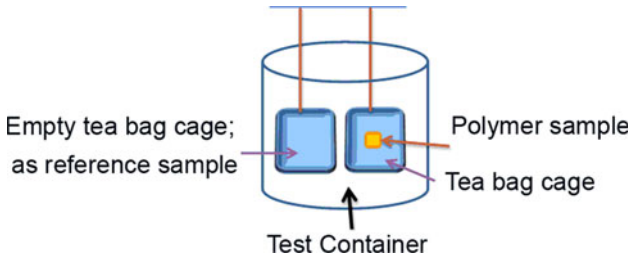


Fig. 2 Schematic presentation of tea bag test method; Polymer samples were immersed or buried in selected environment using tea bag like cages made of inert non-degradable plastic nets. The method resembles method described in (1) Swelling capacity http://en.wikipedia.org/wiki/Swelling_capacity, (2) SUPERABSORBENT POLYMER WIPO Patent Application WO/2003/018072 March 06, 2003 http://www.devileye.net/catalog/adapted_electrically_conductive_layer/superabsorbent_polymer.html

degradation weight was measured and weight loss of each polymer sample was obtained using the following formula:

$$\text{Weight loss } (\%) = \text{Wt}_1 - \text{Wt}_2/\text{Wt}_1 \times 100 \quad (1)$$

where Wt_1 is the pre-degraded dry weight of the polymer and Wt_2 is the dry weight of the sample after degradation, each time.

After 4 months' degradation, they were thoroughly characterized with various methods including the determination of weight changes, functional group content (FT-IR), molecular weight (GPC), thermal (DSC, TGA) and morphological (SEM, XRD) properties.

Degradation test in river water

PEUU film was immersed in water obtained from Zayande-Rood River in Isfahan, in one container, together with a reference sample (an empty plastic tea bag). Every 15 days the film was removed from container, washed with distilled water, dried under reduced pressure and weighted.

In order to determine if simple hydrolysis of polymer is responsible for the observed degradation of PEUU, a degradation study of the polyurethane in pure water was performed as well. This study was conducted by immersing PEUU sample in pure water according to the aforementioned method.

Degradation in activated sludge

Degradation in an activated sludge was carried out in a manner similar to the procedure described above using an activated sludge instead of river water. The activated sludge was obtained from Municipal waste water in Isfahan.

Soil burial degradation test

PEUU film was buried in soil enriched with plant fertilizer in one container together with a reference sample (an empty inert plastic tea bag). The relative humidity was kept ca. 80 % by Municipal water. Weight loss was measured using the aforementioned procedure.

Water uptake experiments

Water uptake studies were conducted by immersing polymer films (0.05 g) into 25 ml of deionized water kept for 1 and 4 days in an incubator at RT. The samples were removed from water at predetermined time intervals, wiped gently with filter paper and weighed with an analytical balance. The sample mass change resulting from the water uptake (expressed as a percentage) was calculated according to the following formula:

$$\text{Water uptake } \% = M_w - M_d/M_d \times 100 \quad (2)$$

where M_d and M_w are the masses of dry and wet samples, respectively.

Study of toxicity effect of polymer film on microorganism cells

The toxicity effect of polymer was studied on Gram-negative and -positive bacteria. For this, some selected bacteria were grown in Petri dishes on Nutrient agar. Once the growth medium in the Petri dishes was inoculated with the desired bacteria, the plates were incubated at 37 °C in the presence of small pieces of polymer films (small discs with a diameter of 5 mm). The medium comprised a basal medium (the final pH was 7.2) supplemented with PEUU film. All slides were thoroughly cleaned with sterile de-ionized water and sterilized with 70 % ethanol, sonicated for 5 min and rinsed in sterile de-ionized water three times. After 24–72 h, growth of bacteria was investigated under microscope.

Study of the effect of polymer film as nutrition source for bacteria

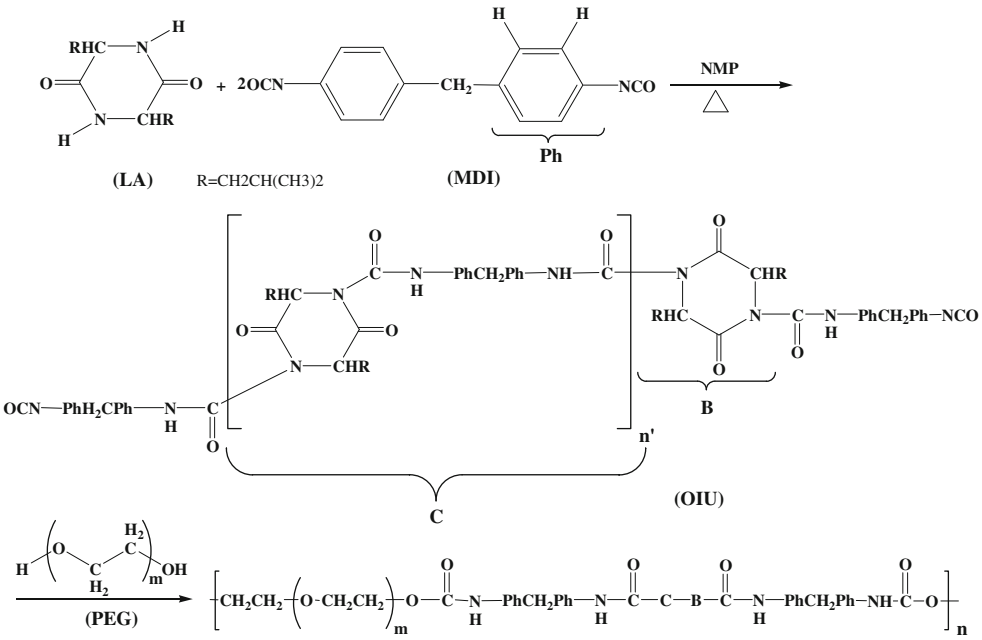
Some selected bacteria grew in test tubes that have a medium comprised of a basal medium (the final pH was 7.2) supplemented with PEUU as the only carbon source for bacteria growth. The test tubes were incubated at 37 °C. After 24–72 h and after 1 week, the growth of bacteria was investigated under microscope.

Results and discussion

Synthesis of PEUU based on amino acid anhydride

LA as an amino acid-based monomer was prepared via heating L-leucine in dried EG and recrystallization from hot

Scheme 1 Synthesis of PEUUs based on MDI-LA-PEG by two-step polymerization method



ethanol (Rafiemanzelat and Abdollahi 2010). The chemical structure and purity of the resulting compound (LA) were confirmed with FT-IR, NMR, CHN, melting point measurement and thin-layer chromatography (TLC). Then LA was reacted with MDI to establish NCO terminated hard segment containing urea linkage. In the second step, PEG-1000 was added as soft segment and reaction was continued, affording PEG-based PEUU multiblock copolymers whose oligo (Imide-urea) (OIU) hard segment blocks connected with urethane linkages (Scheme 1). The effect of polymerization conditions such as reaction time and temperature, reaction solvent, reaction catalysts and soft segment length on the physical properties, viscosities and yields of the resulting PEUUs was studied in our previous work (Rafiemanzelat and Abdollahi 2010). Our studies showed these polymers based on PEG polyols and amino acid anhydride (LA) were hydrolysable under accelerated acid-catalyzed conditions (Rafiemanzelat and Abdollahi 2010). Here the effect of environmental degradation agents in soil, river water and sludge on these polymers was studied.

Study of changes in PEUUs properties under in vitro degradation tests

Weight loss measurement

In vitro degradation tests were carried out in accordance with methods outlined in "Experimental section". The weight loss percentages of PEUUs followed with exposure time in different environments as shown in Fig. 3. Degradation curves of polymer samples show relatively the same manner. First, after an initial induction period the weight loss percentage values of PEUUs increased with exposure time during 30 days. This could be due to degradation of amorphous regions of polymer samples (step 1). Then curves show a slow degradation rate between 30 and 60 days comparing with step 1. Slow degradation rate during step 2 can be due to crystalline regions and/or interconnected hard segment region in PEUUs. Degradation curve between 60 and 120 days continued with relatively the same slope for soil-buried sample. However; degradation curves show increased slopes for water-immersed samples after 90 or 120 days and continued to 150 days (step 3). The observed differences between degradation rates of two environments (soil vs. water) can be due to better penetration of degradation agents into the polymer bulk because of fluid character and easy penetration of water. Percentage weight loss values of PEUUs increased

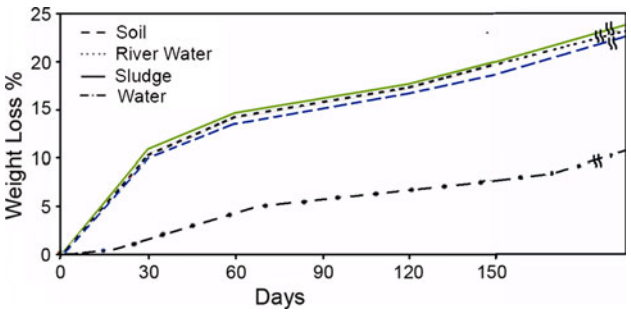


Fig. 3 Comparing the percentage weight loss of PEUUs versus exposure time in soil, river water and activated sludge during 150 days. Weight loss % = $Wt_1 - Wt_2/Wt_1 \times 100$, in which Wt_2 and Wt_1 are weights of polymer sample measured after and before exposure to different environment for every 15-day intervals of weight measurements

with exposure time and varied in the range of 8–12, 13–16 and 16–19 % during degradation steps 1–3 after 30, 60 and 120 days, in soil, river water and sludge, respectively.

Our studies showed that PEUUs based on MDI, LA and PEG-1000 biodegraded at a rate of 10.5 % per week in activated sludge, 10.3 % per week in Zayand-e-Rood river water, 10.2 % per week in land field soil, during the first 30 days of exposure. The polymer films immersed in activated sludge showed 11.3, 18.7 and 20 % weight loss in 30, 120 and 150 days, respectively. Soil-buried samples showed 10.19, 16.2 and 18 % weight loss in 30, 120 and 150 days, respectively. River water-deployed films showed 10.7, 17.8 and 19.5 % weight loss in 30, 120 and 150 days, respectively.

Traditionally, it is believed that amorphous phase is more susceptible than crystalline phase to degradation. It was shown that the degradation of PUs and their copolymers depends on their chemical structure, molecular weight, composition and morphology of polymers, soft segment or hard segment length, phase concentration and crystallinity. Usually PU hard segments provide materials with extra strength due to hydrogen bonding involving urethane and polar groups. Thus hard segment generally degrades slower than soft segment. However, a crystalline soft segment degrades slower than an amorphous soft segment. Thus it can be said that degradation rate can be in the order of: amorphous region of soft segment > crystalline region of soft segment > hard segment. It can be said that ether, urethane and peptide linkages in soft and hard segments of polymer chains may be the points that are susceptible to in vitro degradation and depending on their inter-chain cohesiveness they are attacked by microorganisms with different rate.

Our previous studies showed these PEUUs were hydrolysable under accelerated acid-catalyzed conditions (Rafiemanzelat and Abdollahi 2010). In order to verify significance of simple hydrolysis effect on observed degradation of PEUU, degradation of these polymers in pure water was studied as well. It can be seen that after a long induction period of 15 days, weight loss percentage values of PEUUs increased with exposure time during 150 days very slowly. The polymer films immersed in pure water showed only <5 % weight loss after 150 days (Fig. 3). It can be inferred that simple hydrolysis in pure water exerts a minor effect on the observed degradation. On the other hand, weight loss percentage values of polymer films immersed in river water and sludge would have been much higher than that of the observed degradation of soil-buried samples if simple hydrolysis had played a significant part in degradation.

Scanning electron microscopy (SEM) study

Polymer films were examined under a SEM before and after exposure to degradation tests. It was seen that under SEM, polymer surface shows more holes and damaging splits after degradation for 4 months. Figure 4 provides evidences for progress of degradation process through polymer surface in different degradation environments. Comparing with the above degradation results, it can be said that surface morphology may have a relationship with polymer degradation.

WAXS studies

WAXS analysis was carried out for PEUUs before and after degradation test (Fig. 5). The percentage of crystallinity was calculated through graphical integration of the diffracted intensity data in the 2θ range 5–60° and subtraction of the amorphous scattering band intensity. Diffraction patterns for PEUU-based PEG-MDI-LA shows two main reflection patterns A and B before exposure to soil with maxima at about $2\theta = 6.5$ and 20° (Fig. 4a). It can be suggested that the crystalline phase which develops at A region in PEUUs is associated with crystallization of MDI-LA hard segment. Crystalline domain of hard segment and PEG-MDI are distributed in amorphous phase overlapping with broad diffusion scattering patterns at B. After degradation in soil, the region B shows a drastic decrease in crystallinity and is changed to a broad diffusion

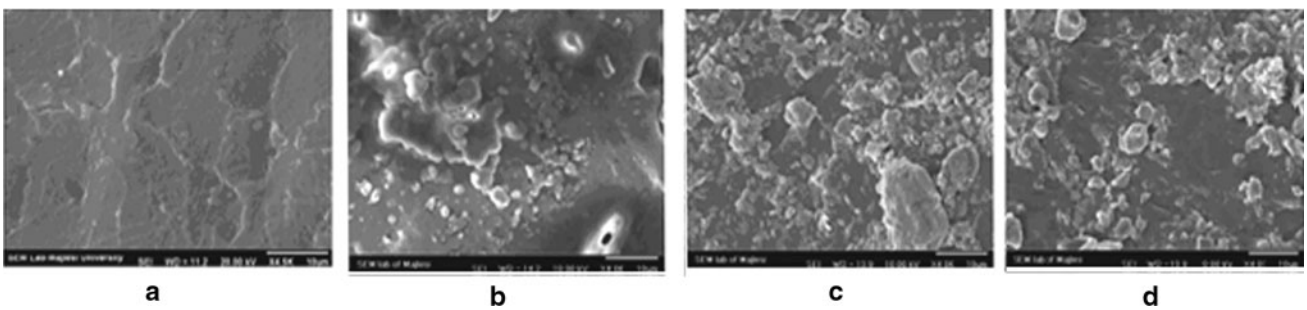


Fig. 4 Scanning electron micrographs of PEUU surface before (**a**) and after exposure to river water (**b**), activated sludge (**c**), soil (**d**) at RT for 4 months

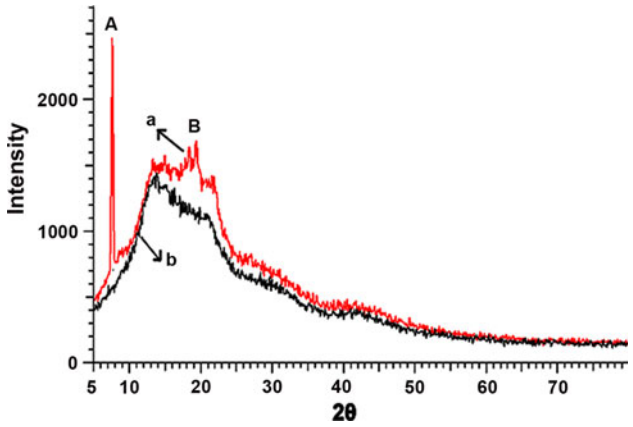


Fig. 5 WAXS spectra of PEUU based on PEG-1000 before (*a*) and after (*b*) exposure to soil for 4 months

scattering (amorphous halo) with maximum at $2\theta = 15°$ and the region A vanishes. Diffraction patterns obtained for PEUU before exposure to soil showed 9 % of crystallinity at A region. Diffraction patterns obtained for PEUU after exposure to soil show 2.3 % crystallinity at B region, and A region has been removed. As mentioned earlier, hard segments generally degrade slower than soft segments and amorphous phase is more susceptible than crystalline phase to degradation. However, comparing the crystallinity of polymers before and after exposure to soil shows that the total percentage of crystallinity of polymers decreased after degradation test and some crystalline regions associated with hard segment have disappeared. River water and sludge degradation tests also show the same results. These data give us an idea about degradation of crystalline phase together with amorphous phase. It can be inferred that a selective degradation preferably has arisen in crystalline regions of polymers affecting both soft and hard segments. This could be associated with the effect of microorganisms presenting in these environments. Our previous study showed that the percentage of crystallinity of polymers increased after hydrolytic degradation test arising from washing out amorphous phase more than crystalline phase (Rafiemanzelat and Abdollahi 2010). Our finding in this study shows that both soft and hard segments, crystalline regions in addition to amorphous regions, have been degraded. Decreasing polymer crystallinity after tests shows the effects of biological and environmental degradation agents as well as hydrolytic, chemical and physical degradation agents on polymer degradation.

Water uptake

For biological environmental degradation, the amount of penetrated water molecules into the bulk of polymers plays an important job on their hydrolytic and microbial degradation. Data of water uptake percent as a function of time show that the equilibrium water uptake increases with increasing time and reaches constant value after 72 h. This behavior is due to the new hydrogen bonding interaction between water molecules and PEG segments or polar hard segments of PEUU. Water uptake percentages for PEUU were 40.9 and 58.7 % after 24 and 72 h, respectively.

Thermal properties of PEUU

Thermal properties of PEUU before and after degradation tests were evaluated with TGA and DSC techniques. DSC and TGA data of PEUU show that these polymers are thermally stable and processable. It can be seen that their T5 and T10 % are about 300 °C, and their T_g and T_m are below their decomposition temperatures.

Figure 6 represents thermal behavior of PEUU samples before and after soil burial degradation test. It can be seen that after 4 months' exposure to soil T_{gs}, T_{gh} (T_m) and $\Delta T_g = T_{gh} - T_{gs}$ of PEUU are higher than those of PEUU before degradation test (Table 1). The aforementioned transitions of PEUU after test show higher depression than those of PEUU before test. It can be said that the residual sample shows more inter-chain interactions due to increasing of hard segment contents because of faster degradation and washing out of soft segment. The remaining of higher-molecular-weight chains more than short chains (GPC confirm this result) can be another reason for increasing thermal stability and thermal transitions. DSC results show that T_{gh} of PEUU after test is higher than T_{gh} of the relevant PEUU before test. This can be due to the greater contribution of hard-segment domains, which decreases chain flexibility and consequently increases T_g.

TGA thermogram of PEUU after degradation test shows increasing of its initial and final thermal stabilities corresponding to T5, T10 % and char yield % (Fig. 7).

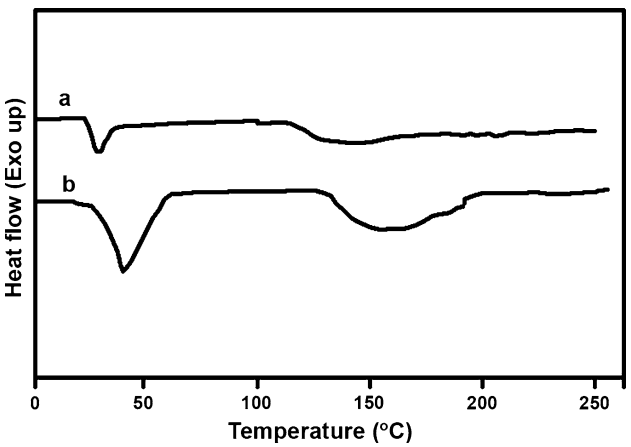


Fig. 6 DSC trace of PEUU under N_2 atmosphere at heating rate of 10 °C/min before (*a*) and after (*b*) exposure to soil for 4 months

Table 1 Thermal stability and thermal properties data of PEUU-based MDI-PEG-LA before and after exposure to soil for 4 months

Char yield%[a]		T10 (°C)[b]		T5 (°C)[c]		T_{gh} (T_m) (°C)[d]		T_{gs} (°C)[e]	
After	Before	After	Before	After	Before	After	Before	After	Before
18	16	345	307	329	283	165[f] (140–190)[g]	140[f] (120–170)[g]	40[f] 32–60[g]	30[f] 28–35[g]

[a] Percentage of weight residue at 600 °C recorded by TGA at heating rate of 20 °C/min in N_2 before and after degradation test

[b] Temperature at which 10 % weight loss was recorded by TGA at heating rate of 20 °C/min in N_2 before and after degradation test

[c] Temperature at which 5 % weight loss was recorded by TGA at heating rate of 20 °C/min in N_2 before and after degradation test

[d] Data range were read at the beginning and at the end of the transition taken from the heating DSC traces at heating rate of 10 °C/min

[e] Data were read at the middle of the transition taken from the heating DSC traces at heating rate of 10 °C/min

[f] Glass transition temperature of hard segment and/or melting temperature of crystalline microdomain of hard segment before and after degradation test

[g] Glass transition temperature of soft segment before and after degradation test

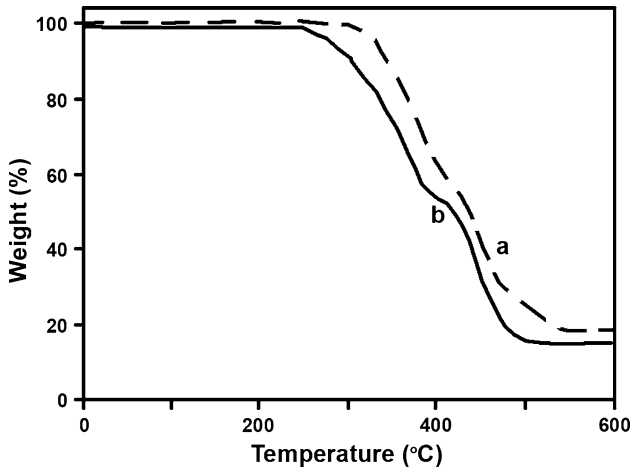


Fig. 7 Comparing TGA thermograms of PEUU under N_2 atmosphere at heating rate of 20 °C/min after (*a*) and before (*b*) exposure to soil for 4 months

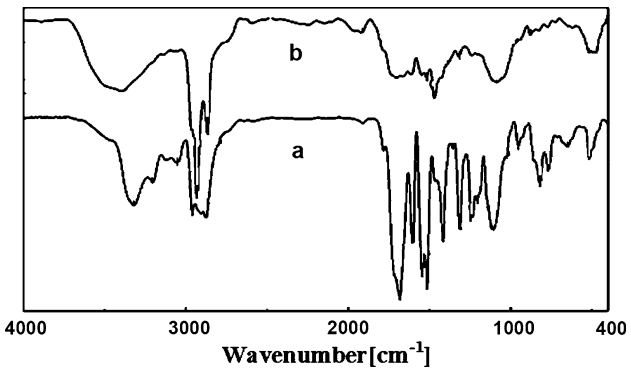


Fig. 8 FT-IR spectra of soil-degraded polyurethane: (*a*) before and (*b*) after soil-burial degradation for 4 months

This confirmed the aforementioned conclusions about increasing contribution of hard-segment content, more inter-chain interactions and average molecular weight of residual polymer comparing with PEUU sample before test. Resulting data obtained from those PEUU specimens exposed to river water and sludge are to some extent similar.

FT-IR studies

FT-IR spectra of PEUU based PEG after soil burial degradation test show reduction or elimination of absorption bands of C=O, C–N, C–O, aliphatic and aromatic C–H, C=C compared with the original sample (Fig. 8). This indicates that the chemical structure of polymer changed after the soil burial test, as a result of the hydrolytic and enzymatic degradation of the polymer chain. It can be said that hydrolytic degradation was responsible for the formation of O–H and amine N–H bonds. This new bonds are highlighted by the appearance of a broad band centered at 3,400 cm^{-1}. The decrease in the bands at 2,930, 1,446 and 1,100 cm^{-1} indicates a possible break and elimination of some segments containing C–O, C–N and aliphatic/aromatic C–H groups. C=O absorption band has been omitted at 1,446 as well. The soil conditions are suitable for the growth of microorganism due to the high humidity, proper temperature of 30 °C and pH 7.5. Microorganisms present in soil are more numerous because of the additional nourishment provided in a soil environment. They can be responsible for hydrolytic and enzymatic degradation of polymer chains at amide, peptide, urethane and ether groups.

Comparing these results with FT-IR spectra of PEUU before (A) and after (B) exposure to hydrolytic degradation, it can be seen that there is not significant difference between A and B spectra (Rafiemanzelat and Abdollahi 2010). In our previous study hydrolytic degradation was responsible for alkoxy group creation, which led to the OH bond formation at 3,400 cm^{-1} as shoulder. The new carbonyl function was observed by C=O peak broadening

from 1,700 to 1,670 cm^{-1}, and a decrease in peak intensity of the CH_2 function was observed at 2,960 cm^{-1}. The decrease in the bands at 1,117 and 1,042 cm^{-1} indicated a possible break in the C–O bond of the urethane or ether group (Rafiemanzelat and Abdollahi 2010). Thus it can be said that there is a great difference between FT-IR spectra of environmentally degraded polymer and hydrolytically degraded polymer.

Study of toxicity and nutrition effects of PEUUs

The toxicity effect of the copolymer films was evaluated by incubating the *E. coli*, *Staphylococcus aureus* and, *Micrococcus* bacteria in the presence of copolymer films over a period of 24–72 h or 7 days at 37 °C.

The aim of this experiment was to simulate clinical conditions to determine the potential toxicological hazard of the copolymer films on bacteria without affecting the chemical or mechanical properties of the copolymer films. The films did not show toxicity against *E. coli* and *Staphylococcus aureus* bacteria. The absence of inhibition zone of growth on Nutrient agar-based medium and dead bacteria around polymeric films showed nontoxic behavior of this polymer. Figure 9 shows the polymer film in contact with the bacterial cells with inhibition zone around the PEUU samples was not formed.

Study of the growth of these bacteria in a growth medium comprising a basal medium and copolymer films as the only carbon source for bacterial growth shows biofilm formation on polymer surface by Micrococcus (Fig. 10). After initial attachment of microorganisms to surfaces of polymer, their growth can form extensive biofilms and damage the materials.

These data support the hypothesis that the degradation of the polymer film is caused by enzymatic effects of microorganisms existing in soil, river water, and sludge and this PEUU shows no toxic effects on those microorganisms. Biofilm formation on polymer surface supports the aforementioned assumption. Study of toxicity effect of the copolymer films or its safety for human beings, as well as cytotoxicity tests utilizing mammal cells are ongoing in our lab.

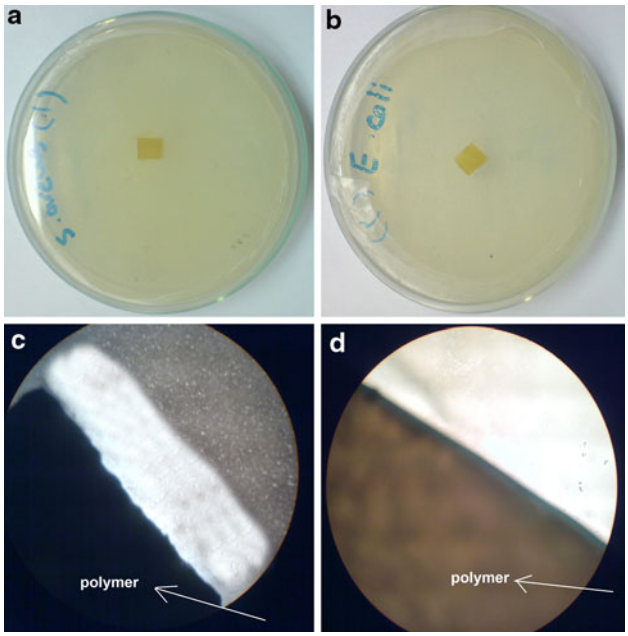


Fig. 9 PEUUs films in cell culture plates (**a**) polymer film in *Staphylococcus aureus* cell culture plate, (**b**) polymer film in *E. coli* cell culture plate. Light microscopy of PEUU films after exposure to the *Staphylococcus aureus* (**c**) and *E. coli* (**d**) bacteria after a period of 24 h

GPC analysis

The weight average molecular weight (M_w) and number average molecular weight (M_n) of the PEUU samples were determined using a gel permeation chromatography system before and after degradation test. A typical GPC chromatograph of PEUU sample shows to some extent a bi-modal peak (A, B regions) in GPC chromatograph

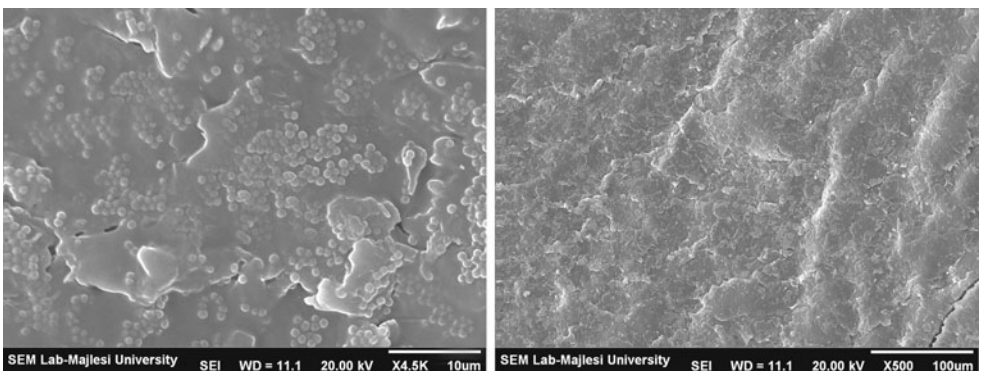


Fig. 10 SEM micrographs of biofilm formation of *Micrococcus* bacteria on polymer surface after 7 days' incubation at 30 °C and 60 % relative humidity

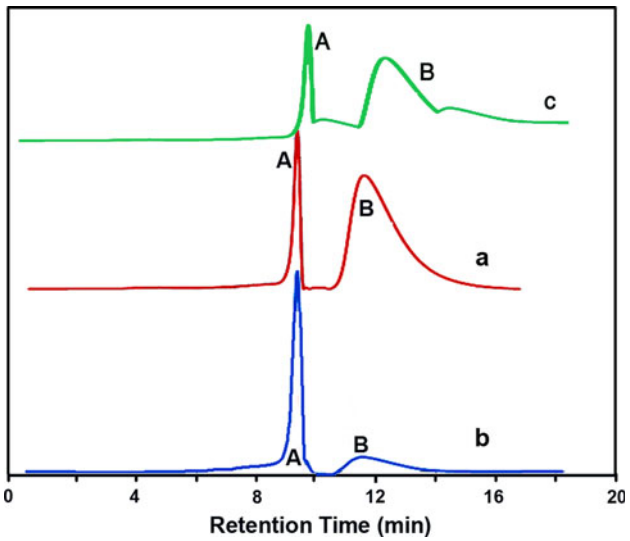


Fig. 11 GPC curves of PEUU before (*b*) ($M_w = 8.357 \times 10^3$, $M_n = 7.924 \times 10^3$) and after degradation in soil for 4 months (*a*) ($M_w = 8.005 \times 10^3$, $M_n = 7.718 \times 10^3$). Increasing the time available for degradation in soil, up to 6 months. Region B with maxima at M_n = 8,500 and region A with maxima at M_n = 3,450

before degradation test, corresponding to molecular weights of about 2,800 and 7,900 (Fig. 11b). The GPC profile after degradation shows a bi-modal profile (A, B) corresponding to molecular weights of about 2,900 and 7,700 (Fig. 11a). It can be seen from the GPC profile that PEUU typically degraded within 4 months breaking down into shorter fragments. This resulted in increasing of peak intensity at lower average molecular weights side at regions B and overall decrease in M_w and M_n. Thus PEUU backbone consists of the hydrolytically and biologically degradable bonds.

GPC peak profile of PEUU at A region corresponding to lower average molecular weights chains shows an increase in average molecular weights after 4 months' exposure to soil. It can be said that remaining chains at region A comprises of higher molecular weight and/or higher crystalline components of polymer residue.

Comparing with GPC curve before degradation test, region A shifts to higher average molecular weights giving us an idea about degradation and washing out of lower molecular weights of polymer chains and remaining hydrolytically and biologically more resistant components. However; region B shifts to lower average molecular weights, suggesting degradation and fragmentation of higher molecular weights of polymer chains. Thus it can be said that during the available period for degradation course (4 months), degradation agents in soil were able to fragment and remove lower-molecular-weight chains (A region), while they had only a chance to fragment higher-molecularweight chains into lower-molecular-weights chains (B region). This hypothesis can be proved by increasing the time available for degradation, for example, up to 6 months. It can be seen that after fragmentation of polymer chains in region B, the resultant low-molecular-weight chains washed off from the polymer bulk. Thus region B shifted to higher average molecular weights and showed a multi-modal peak with maxima at M_n = 8,500 (Fig. 11c). The same is true for region A with maxima at M_n = 3,450. After this period, degradation rate increased which resulted in increasing slop of weight loss versus time curves. It is expected that higher molecular weight and higher crystalline components of polymer chains remain with increasing degradation time.

It should be mentioned that after further purification and fractionation of polymer sample, mono-modal GPC peak corresponding to A region or B region was obtained. However, the same GPC patterns for degradation route of A or B region was observed (to some extent the same as Fig. 11a–c). It means increasing average molecular weights with increasing exposure time and multi-mode peaks were observed. On the other hand, these GPC curves' behaviors of the first polymer sample and fractionated samples interestingly confirm offered discussions about the effect of polymer morphology and molecular weight on degradation (Kumar et al. 2003; Chandra and Rustgi 1998). Long chains should be broken down to shorter chains and short chains are more susceptible than long chains to degradation hydrolytically or biologically.

Conclusion

In this study degradable PEUUs based on a hard segment containing cyclopeptide and PEG-1000 as soft segment were synthesized in NMP. Their degradation in environment was studied in soil, river water and activated sludge. Significant changes in their physical and morphological properties as well as their percentage weight loss and functional group alterations were proved by DSC, XRD, FT-IR, GPC and SEM data. XRD study showed that crystalline peak associated with hard segments was removed after degradation test confirming degradation of hard segment based on cyclopeptide. The SEM micrographs of degraded polymer films showed great number of small holes, cracks, cavities and surface irregularities indicative of hydrolytic and biological attack on polymer surface. Study on toxicity and nutrition effects of polymer films demonstrated that these PEUUs do not show toxicity against bacteria. Biofilm formation on polymer surface by Micrococcus bacteria proved initial attachment of microorganisms to surfaces of polymer, their growth and damaging effect on the polymer. This supports the hypothesis that the degradation of the PEUU film is caused by enzymatic effects of bacteria existing in environment. Water uptake percentage data as a function of time showed good

water penetration into the polymer bulk because of hydrogen bonding interaction between water molecules and hydrophilic PEG segments or polar hard segments of PEUU. Thus it can be said that these cyclopeptide-based polymers containing hydrophilic PEG soft segments could be considered as environmentally degradable polymers. In addition these polymers are thermally stable, soluble in polar organic solvents and are solution and thermally processable.

The effect of soft segment type, soft segment length, polymer molecular weight and polydispersity, film preparation conditions and….on the polymer degradation in different conditions in the presence of different degradation agents are being studied in our lab.

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