Synthesis and characterization of novel biodegradable water dispersed poly(ether-urethane)s and their MWCNT-AS nanocomposites functionalized with aspartic acid as dispersing agent

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Abstract

New biodegradable poly(ether-urethane)s (PEU)s were synthesized via the reaction of *L*-leucine anhydride cyclo-peptide, polyethylene glycol-1000 and hexamethylene diisocyanate. Then, they were end-functionalized with aspartic acid (AS) as a dispersing agent and were dispersed in water. MWCNTs were also functionalized by AS under microwave irradiation. Polymer/MWCNT-AS composites and polymer/MWCNT-AS water dispersed composites were prepared through an ultrasound-assisted method. We have designed these PEUs with two different structural architectures (PA1 and PA2) which can be readily dispersed in water (PA1-D and PA2-D). The structure and properties of the polymers, MWCNTs-AS and PEUs/MWCNTs-AS composites were investigated by FTIR and NMR spectroscopy methods and FE-SEM, TEM and TGA techniques. The particle sizes of the resulting PA1-D and PA2-D dispersions were in the range of 200–300 nm. The results showed that by increasing MWCNT-AS loading, the degradation rate and particle sizes of the dispersed composites decreased, while thermal stability and dispersion stability of the composite systems increased. The degradation tests of polymers and their composites in PBS at 37 °C after 10 days showed weight losses ranging from 23 to 44 and 17–37%, respectively. The cytotoxicity study of polymers using the direct-contact test on L929 mouse fibroblast cell line showed no toxicity. Other properties such as thermal stability, dispersion's particle size, degradation rate and morphology of the composites were studied, and the effect of simultaneous dispersion of MWCNTs-AS and PEUs in water on the properties of the resulting mixtures was studied. We suggest that these polymers have tunable properties which may potentially be considered for drug carriers' studies.

Keywords Copoly(ether-urethane) · Water dispersion · *L*-leucine anhydride cyclopeptid · Aspartic acid · Bio-functionalized MWCNT · Biodegradable

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Introduction

For their excellent physicochemical properties, polyurethanes (PU)s have been extensively used in biomedical applications [1–3]. Some of these outstanding applications include heart valves, aortic grafts, pacing lead insulation, catheters, temporary scaffolds and controlled release systems of active pharmaceuticals [4].

One of the benefits of PUs is the possibility of synthesizing them using various subunit components. PUs are an arrangement of alternating hard and soft segments, and by varying their chemical compositions, their properties can be tuned [5]. This strategy enables us to create different chain architectures which are applicable in manufacturing drug delivery systems such as micelles, hydrogels, nanoparticles and microsphere, as well as different release triggered systems. It is easy to insert the hydrophilic

and hydrophobic segments into the PU chains to interact with both hydrophilic and hydrophobic drugs [6–8]. There are many examples of different PU drug carriers. For instance, Zhang et al. synthesized the amphiphilic poly(lactic acid)-polyurethane having carboxylic groups via step polycondensation reaction. They prepared PULA micelles and studied the degradation profile of micelles. They showed the effect of the carboxylic acid array of the chains on the degradation rate and the drug-releasing rate [9]. Reddy et al. synthesized segmented poly(urethane-urea) based on aliphatic diisocyanate 2,6-diisocyanato-methyl caproate(lysine-based diisocyanate) with poly(ϵ -caprolactone diol)s. They also studied degradation of these polymers in alkaline solution and in vitro drug release of sulfamethoxazole in pH 7.4 buffer [10]. Another example in this field is a novel pH-sensitive multifunctional polyurethane micelles having hydrazone linkers which were acid-cleavable. The synthesized poly(ϵ -caprolactone)-hydrazone-poly-(ethylene glycol)-hydrazone-poly(ϵ -caprolactone) diol (PCL-Hyd-PEG-Hyd-PCL) could be a good choice as a multifunctional biodegradable micelle [11].

Due to the control of the release of volatile organic compounds (VOC), preparation of waterborne PUs (WBPU)s has been one of the most economical and environmental-friendly fields of polymerization [12]. WBPU)s can be synthesized either in non-ionomer or ionomer forms. Non-ionomer types contain hydrophilic soft blocks such as PEG, whereas the ionomer types contain pendant acid [commonly dimethylol-propionic acid (DMPA)], or tertiary amine groups, which are neutralized to form carboxylate or ammonium salts [13].

The biodegradability of PUs is generally achieved by incorporating labile and hydrolysable functional groups into the polymer backbones [14]. It has been shown that copolymers consisting of PEG and peptide sequences are highly water-soluble, degradable, non-immunogenic and relatively non-toxic [15]. The hydrophilic nature of PEG and its derivatives results in avoiding absorption by serum proteins and prevents recognition of the micelles by the cells of the reticuloendothelial system after intravenous injection. This is an undeniable benefit of PUs containing PEG derivatives for biomedical usages [16]. Diisocyanates and chain extenders which are typically used as the hard segment in PUs are not biocompatible. These compounds produce toxic and carcinogenic compounds such as aromatic diamines after degradation. Therefore, PUs based on aliphatic diisocyanates or bio-based diisocyanates are preferred for in vivo applications [17–20].

Recently, the synthesis of polymers containing amino acids residue has been an interesting field of study. It has been shown that the presence of amino acid functionality can lead to polymers with improved solubility, biocompatibility and biodegradability [21].

2,5-Diketopiperazines are subject of interest due to their biological properties such as the inhibition of plasminogen activator-inhibitor-1 and alteration of cardiovascular and blood-clotting functions [22–24]. They have also been interesting as anti-tumoral, antiviral, antifungal and antibacterial agents [25–27]. Some of the chemical properties of 2,5-diketopiperazines such as resistance to proteolysis, simulating of peptidic pharmacophoric groups, donor and acceptor properties for hydrogen bonding and favoring interactions with biological targets are very interesting for pharmaceutical chemistry studies [28, 29]. Amino acid anhydrides prepared from natural amino acids are kinds of 2,5-diketopiperazines, which may be good candidates for the synthesis of polymers with interesting biodegradability and bioactivity properties [30, 31].

Carbon nanotubes (CNTs) have recently been raised as one of the most advanced nano-carriers for the highly efficient delivery of drugs and biomolecules. They can be conjugated covalently or non-covalently with drugs, biomolecules and nanoparticles. It has been shown that functionalized CNTs exhibit very low toxicity and are not immunogenic. Thus, they could be motivating carriers with an excellent capability for the development of a new-generation of drug delivery systems [32–34]. On the other hand, dispersing CNTs in a polymer matrix is one of the most critically essential features in preparation of CNT/polymer nanocomposites. One of the considerable improvements in the dispersion of CNTs in a polymer matrix can be attained through bio-functionalization of CNTs. This can lead to the better dispersion of CNTs in water, as well as in polymer matrix [35–37].

In this work, *L*-leucine anhydride cyclo-peptide (LAC), as an amino acid, and 2,5-diketopiperazine based monomer together with PEG-1000, which have suitable properties for the preparation of non-immunogenic and biodegradable polymers, are utilized to synthesize nontoxic and biodegradable polymers. Using hexamethylene diisocyanate (HDI), as an aliphatic diisocyanate, it is expected to prevent forming toxic compounds such as aromatic amines which are formed after the degradation of the aromatic diisocyanates. In addition, it was anticipated that the application of AS instead of DMPA, as the ionic dispersing agent, played a vital role in the bio-functionalization of the chains for dispersing them in water. We used multiple strategies, such as surface functionalization of MWCNTs and ultrasonication solution blending procedure as fast, green and economical methods, to disperse the MWCNTs-AS in the polymer matrix. Furthermore, compatibility of these functionalized PEUs as a polymer matrix for aspartic acid-modified MWCNTs and their simultaneous dispersion in water are discussed in this work, for the first time. The focus of the current work was on the preparation of the polymers, polymer water dispersions, polymer composites and polymer composites water dispersions. Then, the

properties of the polymers and polymer water dispersions were studied and the effects of MWCNT-AS on thermal, degradation and dispersion characteristics of the polymer composites were studied. It was expected that the modification of MWCNTs by AS and the polymer, increased water disperseability of CNTs and mutually MWCNT-AS modified thermal and dispersion characteristics and degradation rate of the polymers.

Experimental

Materials

All chemicals were purchased from Sigma-Aldrich and Merck (Germany). 1,6-Hexamethylene diisocyanate (HDI) (Merck, Germany) was used as received. Poly(ethylene glycol) (PEG) (Merck, Germany, $M_n = 1000$ g/mol) were dried under vacuum at 80 °C for 6 h. Azeotropic distillation in the presence of dried toluene did not show much improvement in drying PEG according to Karl–Fisher test. *L*-Leucine and aspartic acid (AS) (Merck, Germany) were dried under vacuum at 80 °C for 3 h. Ethylene glycol (EG) (Merck, Germany) and *N*-methyl-2-pyrrolidone (NMP) (Merck, Germany) were distilled under reduced pressure over dried BaO (Sigma-Aldrich, Germany). Dimethyl sulfoxide (DMSO) (Merck, Germany) and dimethylformamide (DMF) (Merck, Germany), dimethylacetamide (DMAc) and ethanol together with sodium hydrogen carbonate (NaHCO_3) (Merck, Germany) were used as received. MWCNT (diameter 10–20 nm, length 30 μm , purity > 95 wt%) was purchased from Neutrino Co. (Iran). H_2SO_4 98%, KMnO_4 , and HCl from Merck (Germany) were used for the preparation of oxidized MWCNTs.

Apparatus and measurements

FTIR spectra were recorded on a Jasco FTIR 6300 spectrophotometer (Japan), and vibrational frequencies were reported in wavenumber (cm^{-1}). Band intensities and designations were assigned as weak (w), medium (m), shoulder (sh), strong (s) broad (br), stretching (str) and bending (bend).

^1H NMR (400 MHz) spectra were recorded on a Bruker, Avance 400 instrument (Germany) in dimethyl sulfoxide ($\text{DMSO}-d_6$) at room temperature (RT). Multiplicities of proton resonance were designated as singlet (s), doublet (d), doublet of doublet (dd), multiplet (m) and broad (br).

Inherent viscosities (η_{inh}) were measured by a standard procedure using an Ostwald glass capillary (U-tube) viscometer (UK) at RT and concentration of 0.4 (g/dL) in DMF.

For measuring the particle size and the surface charge of particles (ZP), we used Malvern Zetasizer ZEN 3600 (UK), and all measurements were carried out at RT.

For the assessment of polymer surface, before and after degradation test, atomic force microscopy (AFM) (DME Model, Germany-Dual Scope DS M95) instrument was used. All micrographs were presented in top-view at RT, with no filtering to ensure the quality of images. The samples were finely ground and diluted with methanol, after that, dispersed on a microscope slide and studied by AFM.

The cell cytotoxicity of the samples was assessed based on the ISO-10993-5:2009 standard protocol. Transmission electron microscopy (TEM) images were also provided by a Philips CM 120 microscope (Germany); the instrument was adjusted at 100 kV. The morphologies of the functionalized MWCNTs and PEU/MWCNT-AS were studied using field emission scanning electron microscopy (FESEM). The micrographs were obtained at 15 kV using a HITACHI S-4160 instrument (Tokyo, Japan).

The thermal stabilities of MWCNTs, PEUs and PEU/MWCNT-AS nanocomposites were established by thermogravimetric analysis technique (TGA) (STA503 TGA, Bahr-Thermoanalyse GmbH, Hüllhorst, Germany) in the nitrogen atmosphere (flow rate 60 cm^3/min). A heating rate of 10 °C/min was applied in each experiment.

A Samsung microwave oven (2450 MHz, 900 W) (Seoul, South Korea) was used for the functionalization of MWCNTs. The composites dispersions were prepared using a MISONIX ultrasonic XL-2000 SERIES (Raleigh, North Carolina, USA) instrument. Ultrasonic irradiation was performed with the probe of the ultrasonic horn being immersed directly in the solution with the frequency of 2.25×10^4 Hz and the power of 100 W.

Synthesis of PEUs

PEU copolymer with one aspartic acid-end cap (methodA1)

A typical preparation method of PEUs, by a three-step polymerization route was as follows: in a dried 25 mL two-necked round bottom flask equipped with a N_2 balloon and magnetic stirrer, PEG (0.442 g, 4.420×10^{-4} mol) and NMP (0.30 mL) were stirred at 40 °C for 10 min to obtain a homogeneous solution; then HDI (0.142 mL, 8.840×10^{-4} mol) was added. The solution was heated between 40 and 60 °C for 1 h.

Then, the reaction mixture was heated up to 70 °C and kept at this temperature for 2 h, then was heated and kept at 80 °C for 4 h and finally the temperature was reached to 90 °C and kept for 8 h. Afterwards, LAC (0.100 g, 4.420×10^{-4} mol) which was synthesized by the same

procedure reported earlier [31] and 0.4 mL NMP were heated up to 120 °C and added to the reaction mixture. The temperature was kept at 120 °C for 10 min to dissolve LAC completely. Then, the temperature was reduced to 60 °C, and the reaction was preceded at this temperature for 1 h.

After this step, the reaction was kept at temperature between 80 and 100 °C over a period of 9 h. Then, the temperature was reduced to 60 °C, and a solution of AS (0.058 g, 4.420×10^{-4} mol) in hot DMSO (1 mL) was added to the reaction mixture (AS is dissolved in DMSO better than in NMP). Subsequently, the reaction mixture was heated further at 80 °C for 4 h and at 90 °C for another 4 h. Then, the viscous solution was poured into 5 mL distilled water when FTIR monitoring showed NCO consumption by fading its peak at 2270 cm^{-1} .

After shaking and stirring for 30 min, the precipitated polymer was isolated by filtration, dried at 50 °C for 2 h under vacuum to give 0.155 g (21%) of the polymer. The inherent viscosity of this polymer was 0.40 dL/g in DMF, and we evaluated its chemical structure by FTIR and ^1H NMR techniques.

FTIR absorption bands (cm^{-1}) for PA1, (KBr): 3432 (m, br) NH amid str., 3332 (m, br) NH urea str., 3254 (m) NH urethane str., 3060 (m, br) carboxylic acid O-H str., 2925 (m, br), 2857 (m, br) CH aliphatic str., 1772 (m) C=O urethane str., 1721 (s) C=O acid str., 1676 (s) C=O urethane-amid str., 1618 (m, sh) C=O urea str. 1445 (m) and 1363 (s) C–N str. 1070 (m), 945 (w) C–O–C ether and urethane str., 814 (w) NH bend., 764 (m) O=C–O, 621 (m), 566 (w), 490 (w).

^1H NMR peaks, DMSO- d_6 at RT, (δ , ppm): 1.2 (d, CH_3 (a1), 6H), 1.51 (t, CH_2 (a2), 2H), 1.80 (m, CH(a3), 1H), 2.8 (dd, m, CH_2 (b'), 2H), 3 (t, CH_2 (b), 4H), 3.8 (t, CH(c), H), 4.8 (m, CH (d'), 1H), 5.1 (t, CH_2 (d), 4H), 6.5–6.9 (br, NH urethane(e)), 9.4–9.5 (br, NH urea (e')), 11.4 (br, OH acid(f), 2H).

PEU copolymer with two aspartic acid-end caps (method A2)

A typical preparation method of PEU, by three-step polymerization method was as follows: In a dried 25 mL two-necked round bottom flask, PEG (0.442 g, 4.420×10^{-4} mol) and NMP (0.30 mL) were mixed by a magnetic stirrer at 40 °C for 10 min to obtain a homogeneous solution, then HDI (0.142 mL, 8.840×10^{-4} mol) was added. The solution was heated between 40 and 60 °C for 1 h. Then the reaction mixture was heated at 70 °C for 2 h, at 80 °C for 4 h and finally at 90 °C for 8 h. Afterward, LAC (0.050 g, 2.210×10^{-4} mol) and 0.4 mL NMP were added at 120 °C, the temperature was kept at 120 °C for 10 min to dissolve LAC. Then, the temperature was reduced to 60 °C, and the reaction was preceded at this temperature for 1 h.

Then, the reaction was heated between 80 and 100 °C over a period of 9 h. After that, the temperature was reduced to 60 °C, and a solution of AS (0.058 g, 4.420×10^{-4} mol) in DMSO (1 mL) was added into the reaction mixture. Subsequently, the reaction mixture was heated further for 4 h at 80 °C and for another 4 h at 90 °C. Then, the viscous solution of the reaction mixture was poured into 5 mL distilled water. After shaking and stirring for 30 min, the precipitated polymer was isolated by filtration, dried at 50 °C for 2 h under vacuum to give 0.147 g (21.1%) of the polymer. The inherent viscosity of this polymer was 0.42 dL/g in DMF, and we evaluated its chemical structure by FTIR and ^1H NMR techniques.

FTIR absorption bands (cm^{-1}) for PA2, (KBr): 3440 (m, br) NH amid str., 3322 (s, br) NH urea str., 3259 (m, sh) NH urethane str., 3101 (m, br) OH acid str., 2910 (m, br), 2862 (m, br) CH aliphatic str., 1770 (w) C=O urethane str., 1701 (s) C=O acid str., 1668 (s) C=O urethane-amid str., 1620 (m, sh) C=O urea str. 1437 and 1343 C–N str. 1010 (m), 955 (w) C–O–C ether str., 838 (w) NH bend., 755 (m) O=C–O, 641 (m), 560 (w), 496 (w).

Oxidation of MWCNTs

To functionalize MWCNTs, we used the acidic KMnO_4 solution. First, 2 g of MWCNTs was dispersed in 120 mL of H_2SO_4 solution (4.5 M) and then 2 g of KMnO_4 was added. The solution was heated at 95 °C for about 2 h. Then, the suspension was cooled down and filtered. The obtained solid was washed with water, filtered, re-washed with concentrated HCl to remove the MnO_2 byproduct and then re-washed several times. The obtained functionalized MWCNTs (MWCNT-OX) were washed with water repeatedly until the filtrate became neutral and then dried in air at 110 °C for 18 h.

Functionalization of MWCNTs with aspartic acid

Aspartic acid was covalently attached to MWCNT-OX surface by the reaction of the free amine group of the AS with carboxylic acid groups on MWCNT-OX [38]. For the amidation reaction 20 mL of DMAc, 0.20 g of AS, and 0.3 mL of ethylene oxide were stirred for 2 h at 60 °C. Then MWCNT-OX (0.1 g) was added, and the mixture was sonicated for 3 h. This suspension was then poured into a porcelain dish and heated in a microwave oven with an output power of 700 W up to 120 °C for 17 min. Then, the resulting suspension was cooled to RT and poured into 200 mL HCl solution (5% v/v). The mixture was stirred for 30 min at RT. Then, the mixture was centrifuged and decanted to remove the solvents. The obtained product named as (MWCNT-AS) was characterized by different techniques such as FTIR, TGA, FESEM, and TEM.

Water dispersed polymers

A sample of 0.001 g of PA1 (PA2) was added into a one-necked round bottom flask equipped with a mechanical stirrer. Then, a solution of NaHCO_3 (0.77 M) in deionized water (pH 8) was slowly added under vigorous stirring (8000 rpm) to neutralize COOH groups of the terminal AS. Because AS has $\text{pK}_a = 3.9$ and $\text{pI} = 2.77$, in this condition COOH groups of AS would be neutralized ($-\text{COO}^-$). The mixture was stirred for 3 h at 40 °C, and polymer dispersions (PA1-D or PA2-D) with 0.02 wt% solid content were formed. These polymer dispersions can be purified and separated by centrifuging, and then lyophilized to fine powders. Due to the presence of ionic end functional groups ($-\text{COO}^-$) on the polymer chain termini and hydrophilic PEG segments these powders can be dispersed in pure deionized water again.

PEU/MWCNTs-AS composites and their water dispersion counterparts

The typical procedure for preparing the PA1/MWCNTs-AS composite was as follows: 0.1 g PA1 was dissolved in 5 mL DMF. In other flasks, an amount of MWCNT-AS (0.5, 1 or 2 wt%) was added into 5 mL DMF, stirred for 3 h and sonicated for at least 1 h. Then the two mentioned solutions were mixed. The resulting mixture was continuously stirred for 24 h and then sonicated for 1 h at RT to obtain a homogenized dispersion of MWCNT-AS and PA1. Subsequently, the mixture was cast onto a Teflon mold, and allowed to dry at RT for 24 h to form PA1/MWCNT-AS composite film (Scheme 1).

To prepare PA1/MWCNTs-AS water dispersion composite (PA1/MWCNTs-AS)-D the same procedure as mentioned above was followed. After mixing MWCNT-AS/DMF mixture with the solution of PA1 in DMF, the resulting mixture was vigorously stirred for 24 h and then sonicated for at least 1 h at RT. Then, a solution of NaHCO_3 (0.77 M, pH 8) in deionized water was slowly added to the former solution under vigorous stirring (8000 rpm) to neutralize the COOH groups of the terminal AS. Next, the resulting mixture was stirred for 3 h at 40 °C and sonicated for at least 2 h (Scheme 1). The particle sizes and surface potentials of the composites dispersions were determined using Malvern Zetasizer instrument.

Degradation of polymers and their composites in PBS solution

The hydrolytic degradations of PA1 and PA2 were studied in the phosphate buffered saline (PBS) solution (pH 7.4) at 37 °C, in six replicates [(0.1M) $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ buffered (0.9 wt%) NaCl solution]. In the experiments, 10 mg of each polymer sample was immersed in 10 mL of PBS and incubated in an air-circulation oven at 37 °C. The PBS solution

Scheme 1 Preparation routes of PEU/MWCNTs-AS composite and its water dispersion composite

was renewed every other day. After 10 days, the PBS solution phase was removed, and the remaining particles were washed thoroughly with distilled water. Then, they were dried in a vacuum oven at 40 °C for 2 h for further studies. The polymers were weighed before and after degradation tests. The percentage of weight loss for each polymer was calculated using the ratio of the mass difference before and after degradation to its original mass.

Similarly, the percentage of viscosity loss for each polymer was determined using the viscosity difference before and after degradation to its initial viscosity. Furthermore, to follow the degradation profile in samples, we investigated the FTIR spectrum of the polymer before and after PBS exposure.

The same method was followed to study the degradation of PA1/MWCNTs-AS and PA2/MWCNTs-AS composites.

Particle size and zeta potential

The particle sizes and zeta potentials of the waterborne polymers, PA1-D and PA2-D, and their composite dispersions, (PA1/MWCNTs-AS)-D and (PA2/MWCNTs-AS)-D were determined based on direct light scattering method using the Zetasizer. All particle size measurements were performed in deionized water (pH 8) without dilution immediately after preparation of the polymer dispersions using a He–Ne laser beam at 658 nm with a scattering angle of 130°.

Scheme 2 Synthesis of PEU (PA1) via method A1

Evaluation of cytotoxicity

Cytotoxicity of the prepared polymers was evaluated using direct-contact test with L929 fibroblast cell line which was carried out according to ISO-10993-5-2009 standard at Iran Polymer and Petrochemical Institute. L929 cells were cultured in RPMI 1640 supplemented with 10% FBS, 100 U/mL penicillin and 100 U/mL streptomycin in a CO₂ incubator supplied with 5% CO₂ at 37 °C. The cultivation was conducted for 24 and 48 h. The optical microscope was used for the observation of cell viability and cell growth or cell contact on the tested polymer powders. Before this test method, the polymer sample was purified via the freeze–thaw process and was sterilized by immersing it in ethanol 70% and UV irradiation for 10 min. After 24 and 48 h intervals the culture media of L929 cells on the tested sample were compared with positive and negative controls [39, 40].

Results and discussion

Polymer synthesis

LAC as an amino acid peptide based monomer was prepared via thermal dehydration reaction of *L*-leucine in dried EG as we have reported earlier [31] (Scheme S1 in supplementary file). It was shown that the presence of LAC fragments could influence degradation of the PEUs.

PEUs multiblock copolymers were synthesized according to the reaction procedure represented in Scheme 2 and Scheme S2 via three sequential monomer addition steps.

Our studies showed that polymer segments resulting from the reaction of LAC and HDI (polyurea) were precipitated in water, whereas polymer segments resulting from the reaction of PEG and HDI (polyurethane) were soluble in water. It can be concluded that PEG-HDI segments have hydrophilic characteristics and LAC-HDI segments have a hydrophobic nature. In addition, data obtained from degradation studies showed that polymers consisted of LAC, HDI, PEG and AS amino acid showed more bio-degradation compared with

polymers containing HDI, PEG and AS without LAC. Thus, polymer dispersion in water can be controlled via adjusting hydrophilic and hydrophobic blocks length and blocks distribution along polymer chains. In addition, biodegradation rates of PEUs can be controlled via the amount of incorporation of LAC into the PEUs chains. To summarize, we designed polymer chains so that NCO-terminated soft segments were first established via the reaction of PEG and HDI. Then LAC was added as the chain extender and AS was added as the internal dispersing agent and the functionalizing agent of the polymer chain termini.

PA1 was synthesized through the polymerization reaction of PEG, HDI, LAC and AS, respectively, in NMP via the conventional heating method without any catalyst (Scheme 2). In the first step, the diisocyanate-terminated oligo(ether-urethane)s (intermediate compound A) were prepared as soft segments through the reaction of HDI (2 eq) with PEG-diol (1 eq) (FTIR (Fig. S1, spectrum A) and NCO titration test confirmed it). Then, 1(eq) of LAC was added, and the reaction was continued to afford PEU multi-block

copolymer containing PEG-HDI soft blocks and LAC-HDI hard blocks with one NCO capped at one end (Scheme 2, intermediate compound B). Then, intermediate compound B was further reacted with 1(eq) of AS as dispersing agent. Finally, PEU multi-block copolymer chains with two carboxylic acids and one NH end groups were synthesized.

The available NCO% was about 5.6 wt% for the intermediate compound A, and its average molecular weight determined by end group analysis was about 1498.2 ± 0.04 . The available NCO% was about 1.8 wt% for the intermediate compound B, and its average molecular weight established by end group analysis was about 2310.4 ± 0.08 . These data show that the NCO functionality was about 2 and 1 for intermediate compounds A and B, respectively.

The average molecular weight of PA1 determined by end group analysis was about 2442.5 ± 0.45 , and the available acid number established by titration method was about 46. These data showed that the COOH functionality of PA1 was about 2. PA2 was synthesized through a procedure the same as PA1 except that we inserted two AS groups into the polymer structure as end-capping groups (Scheme S2). The same titration and calculation methods were conducted as above and the results showed that the COOH functionality of PA2 was about 4 (according to NCO titration test based on ASTM D 2572) [41].

In these two structures, HDI together with LAC have been incorporated in the hard segments of polymer chains whereas PEG has formed the soft parts.

Using aspartic acid (AS) as dispersing agent, we could prepare unique structures which can act as pH-sensitive water dispersions. It means that PA1 and PA2 could be dispersed in deionized water in the presence of NaHCO_3 solution (pH 8). Conversely, PA1-D and PA2-D dispersions were precipitated by addition of HCl (0.5 M) solution (pH 6.5).

In this work, we used an amino acid, AS, as the internal dispersing agent that can be expectantly considered as a safe agent with respect to cytotoxicity. Thus, the AS end groups

Fig. 1 FTIR (KBr) spectrum of PA1 polymer

Fig. 2 ^1H NMR (400 MHz) spectrum of PA1 in $\text{DMSO}-d_6$ at RT

can serve as dispersing aids and the disperse-abilities of the polymers will depend on the pH values.

Characterization

The structures of PEUs were determined using FTIR and ^1H NMR spectroscopy techniques. The FTIR spectra of the PEUs showed characteristic absorption bands of PEUs as expected. For instance, the FTIR spectrum of PA1 (Fig. 1) showed the N–H characteristic absorption bands of amide, urea, urethane and acid groups around 3432, 3332, 3254, 3060 cm^{-1} , respectively. The carbonyl groups ($\text{C}=\text{O}$) absorption bands of urethane, carboxylic acid, amide, and urea groups could be seen at 1772, 1721, 1676, and 1618 cm^{-1} , respectively. The stretching vibration bands for the C–N bonds in amide moieties represented two peaks at 1445 and 1363 cm^{-1} . The band observed at 2925 cm^{-1} was related to the stretching vibration of aliphatic C–H, and the stretching band around 1255 cm^{-1} was correlated to N–COO vibration. Ether C–O–C fragment showed two absorption bands around the 1070 and 945 cm^{-1} . Therefore, based on these spectral proofs we confirmed the successful synthesis of PA1 and PA2.

^1H NMR peaks of PA1 in $\text{DMSO}-d_6$ at RT (Fig. 2) showed aliphatic hydrogen of LAC (a1–a3) and HDI (a') in the region of 1–2 ppm. The CH_2 (b) protons of HDI moieties neighboring to NH are observed at 3 ppm. The chemical shifts of CH (c) belong to LAC and CH_2 (d) of PEG appeared at 3.8 ppm and 5.1 ppm, respectively. Urethane

N–H (e) appeared around 6.5–6.9 ppm, and urea N–H (e') protons observed at 9.4–9.5 ppm. CH_2 (b') and CH (d') of AS appeared at about 3 and 4.8 ppm, respectively. Furthermore, we can see the chemical shift of acidic protons of AS at 11.4 ppm (Fig. 2).

Particle size of dispersed PEUs

The average particle sizes of dispersed PEUs (PA1-D and PA2-D) were determined by the Malvern Zetasizer instrument based on the Stokes–Einstein equation (Eq. (1)) as follows:

$$d(H) = \frac{kT}{3\pi\eta D}, \quad (1)$$

where k is Boltzmann's constant, T is absolute temperature, η is viscosity and D is translational diffusion coefficient or velocity of brownian motion.

The results revealed that the particle size of dispersed PA1-D in water (pH 8) was around 315.9 nm and that of dispersed PA2-D was approximately 400.7 nm (Table 1). Related reports about nano-sized carriers used in the drug delivery subjects suggest that the practical sizes of carriers should be less than 300 nm. Then, they indeed pass the physiological barriers as the most effective factor which reduces the therapeutic effect of administering drug [42, 43]. Furthermore, it was demonstrated that in most tumors and infected tissues, large molecules ranging from 10 to 500 nm

Table 1 Particle size and zeta potential data of PEUs

Polymer code ^a	PEG ^b (mol%)	AS ^c (mol%)	Hydrophobic part ^d (mol%)	Block structure ^e	η_{inh}^f (g/dL)	Potential ^g (mV)	Z-ave ^h (nm)
PA1-D	20	20	20		0.40±0.1	-11.41±0.2	315.9±0.2
PA2-D	22.22	22.22	11.11		0.42±0.1	-11.80±0.30	400.7±0.5

^aPolymers synthesized via A1 or A2 methods

^bPercent of mole ratio of PEG to the total moles of monomers (as feed)

^cPercent of mole ratio of As (aspartic acid) to the total moles of monomers (as feed)

^dPercent of mole ratio of LAC as Hydrophobic part to total moles of monomers (as feed)

^eBlock structure in the polymer chain in which each component has represented by a graphical symbol

^fInherent viscosity. For measuring the inherent viscosity, 0.04 g of polymer was dissolved in 10 ml DMF and the flow time of polymer solution passing through a narrow capillary is compared to that of the pure solvent by Ostwald glass capillary (U-tube) viscometer

^gZeta potential of polymers measured by the Malvern Zetasizer instrument

^hAverage of particle size of PA1-D and PA2-D measured by the Malvern Zetasizer instrument

Scheme 3 Suggested PA1-D chains arrangements and their self-assembly in aqueous solution with 100% neutralization by NaHCO_3

in size can leave the vascular bed and accumulate inside the interstitial space by EPR effect [44]. As a result, the sizes of these synthetic PEUs systems are in the acceptable applying range.

Schemes 3 and S3 suggest chain assemblies of PA1-D and PA2-D, respectively, where segments layouts were obtained based on the synthesis methods, order of addition of monomers, monomer ratios, and polymer end functionalities.

As shown in Table 1 and based on the schematic representations provided by scheme 3 and (Scheme S3), the particle size of PA2-D is a bit more than that of PA1-D, and this fundamentally can be related to the higher content of PEG in the PA2 chains which was a little more than that of PA1-D. Thus, when PA2-D is exposed to water, water molecules can diffuse into its chains because of grater hydrophilic parts in PA2-D and its hydrodynamic diameter increased compared to that of PA1-D [45–47]. In addition, the percentage of LAC as a hydrophobic segment in PA2 is lower than that of PA1. Thus, the presence of hydrophobic segments in PA1

chains prevents water diffusion and chain expansion which results in decrease of the sizes of PA1-D particles compared to that of PA2-D polymeric aggregates (Scheme 3).

It can be said that LAC monomers are located in the center of the PA2 chains. Therefore, in an aqueous environment, PA2-D chains fold so that they keep their hydrophobic parts away from water molecules (Scheme S3). In this way, the average size of PA2-D particles increases. In other words, hydrophobic LAC-HDI segments undergo intra-chain contraction and inter-chain association. The aggregated parts are quickly stabilized by hydrophilic PEG chains and AS ionic groups. PEU copolymers self-assemble into aggregates with hydrophobic LAC or LAC-HDI segments as the core and hydrophilic PEG segments and carboxyl groups as the corona. These results showed the effect of synthesis methods, the sequence of addition of monomers and monomers ratios on the polymer chain structures, surface zeta potentials, polymer chain aggregates, particle sizes, segments distributions along chains and water interaction behavior. These

two completely different shaped polymeric scaffolds may be suitable as carriers in various drug delivery systems. They can interact with the hydrophilic and hydrophobic environments and drugs. Depending on the case, they would orient their hydrophilic or hydrophobic segments according to their surroundings.

Zeta potential of dispersed PEUs nanoparticles

The charge of each particulate system is the most critical factor which influences its applications. Therefore, we measured the surface zeta potentials of polymeric PA1-D and PA2-D samples. As we know, zeta potential is a scientific term used for electrokinetic potential. From a theoretical point of view, the zeta potential is the electric charge in the interfacial double layer (DL) at the slipping plane relative to a point in the bulk medium away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the charged particles that move under an external applied electric field [45–47]. Zeta potential is calculated based on the following equation:

$$\zeta = \frac{4\pi\mu V}{E\epsilon}, \quad (2)$$

where V is liquid migration speed, η is the viscosity of the external environment, E is voltage, ϵ is dielectric constant of external environment, and ζ is zeta potential. The results are reported in Table 1 and zeta potential was found to be -11.41 mV for PA1-D and -11.80 for PA2-D.

Based on the results, these particulate systems have potential advantages for being used as the polymeric dispersed carriers. These values of ZP are enough and undoubtedly lead to thoroughly dispersed particles [48–50]. The negatively charged particles are not considered to agglomerate

during micelle formation [51, 52]. The ZP can be attributed to the particle size; that is, the larger the size of dispersed particle the more the speed of movement of surrounding liquid would be [45]. In addition, we measured the conductivity of solutions of PA1-D and PA2-D which were 44.2 mS/cm and 49.3 mS/cm, respectively. As expected, the surface potentials and conductivities would be increased with increasing the size of particles.

In the case of PA2-D, its particle size and percentage of AS residue are more than those of PA1 and PA1-D. Thus, the negative surface potential of PA2-D had increased compared to PA1-D. According to previous reports [45–47], the stability of a dispersed system depends on the level of zeta potential, molecular weight and particle size distribution. Since these two polymers, i.e., PA1 and PA2 almost had the same viscosities and PA2 had larger particle size, it was expected that PA2-D should have less stable particulate dispersions. However, due to its higher surface zeta potential and the higher number of acidic groups on its surface, PA2-D dispersion showed the same stability as PA1-D. Furthermore, both of the polymer dispersions possessed the excellent stability for more than 100 days.

Degradation of PEUs in PBS solution

Table 2 represents the results of degradation studies of PA1 and PA2 in PBS. Indeed, these degradation values are due to the extensive amount of biodegradable bonds that are present in peptide, urethane, amide and urea groups [53, 54].

The results in Table 2 indicated that the degradation rate of PA2 was more than that of PA1. It can be attributed to the higher amount of hydrophilic PEG participants in the PA2 chains. Degradation most probably started from the AS hydrophilic shell at the end of the polymer chains (Schemes 3 and S3). Since PA2 has two AS ending groups, then its degradation rate would be more than that of PA1. Briefly, it can be said that higher amount of hydrophilic parts and their accessibility (PEG and AS) and better diffusion of water molecules among the polymer chains resulted in the higher degradation rate of PA2 compared with that of PA1.

Other possibilities are the peptide bond dissociations in LAC rings and urea bonds dissociation at the connection of LAC to the polymer chains. However, it should be noted that these less hydrophilic parts of the chains are less exposed to water to take part in hydrolysis process considerably [55–57]. Furthermore, the LAC residue, due to its circular structure, needs to break through two bonds to afford the chain dissociation, and this would be more difficult to achieve (Scheme S4). In spite of these statements, comparing the control sample with PA2 showed that the presence of LAC moieties in the polymer backbone has a vital effect on the polymer biodegradation. The

Table 2 The results of degradation study of PEUs in PBS

Measured item	PA1	PA2
Weight loss (%) after 10 days ^a	23.24 ± 0.50	44.44 ± 0.40
Primary viscosity	0.40 ± 0.02	0.42 ± 0.01
Viscosity decrement (%) ^b	12.5	28.57
Hydrophilic part (%) PEG ^c	20	22.22
Hydrophilic part (%) aspartic acid ^d	20	22
Hydrophobic part (%) LAC ^e	20	11

^aWeight loss (%) = $\frac{w_1 - w_2}{w_1} \times 100$

^bViscosity decrement (%) = $\frac{v_1 - v_2}{v_1} \times 100$

^cPercent of mole ratio of PEG relative to the total moles of monomers

^dPercent of mole ratio of As (aspartic acid) relative to the total moles of monomers

^ePercent of mole ratio of LAC as hydrophobic part relative to the total moles of monomers

Fig. 3 FTIR spectra of PA1 sample before and after degradation in PBS solution for 10 days

viscosity changes, in the same trends as weight changes for the two samples, confirmed the chain dissociations mentioned above (Table 2).

Degradation of each polymeric sample was further monitored through changes in their FTIR spectra before and after degradation tests. A substantial attention was paid for each sample, as well as for the blank, to eliminate

possible errors due to non-homogeneity or thickness variations. The FTIR spectra showed that hydrolytic degradation occurs in PA1 and PA2 during PBS exposure. Figure 3 displays the polymers degradation and changes in functional groups using FTIR spectra. Dissociation of LAC residue in urea and peptide bonds produced secondary amine and free carboxylic acids groups. The disappearance

Fig. 4 AFM images of PA1 (a) before degradation in PBS; (b) after degradation in PBS

of carbonyl, $-(C=O)-NH-$ stretching band, belonging to the LAC peptide in FTIR spectrum after degradation confirmed this point (Scheme S4).

In addition, the absorption band of amide N–H bond weakened, and the absorption band of the amine N–H bond was appeared. In addition, during the hydrolysis process, some of the urethane bonds convert to alcohol and amic acid, which further were dissociated to amines and carbon dioxide. This process indeed is another reason for broadening of the N–H peak in the FTIR spectrum. Other absorption bands related to urea linkages have also changed after degradation (Fig. 3). The spectral evidence for aspartic acid changes is apparent by reducing the $C=O$ band intensity of acid group of AS which can be related to the dissociation of AS end groups and polymer degradation. These results confirmed the deterioration of the polymer and supported the decrease in the weight and viscosity of the polymer.

AFM

Atomic force microscopy (AFM) has already been used to study the hydrolytic surface erosion of degradable polymers [58]. We used the AFM technique to study the hydrolytic surface degradation of PEUs in PBS solution, as well. From phase imaging, heterogeneity is evidenced by the two distinct regions, a region that appeared bright (hydrophilic region rich in PEG) and a region that is dark (hydrophobic region rich in LAC). The microstructure of PA1 film before exposure to PBS solution was found to be relatively smooth. Evident changes can be observed on the surface of PA1 film after exposure to the PBS solution (Fig. 4b). After the degradation period, the increase in the bright regions and surface roughness were apparent. This behavior can be due to the surface changes of the polymer as a result of water uptake and more hydrophilic nature of the polymer surface after degradation.

The striking point is due to non-uniform water uptaking, which was intensified in specific domains. This fact probably reveals the presence of micro-domains embedded in the bulk of polymer which contain more hydrophilic units. Based on these observations it is evident that water penetration started through more hydrophilic domains. After water penetration and hydrolytic degradation, in which urethane, urea, and peptide bonds were cleaved to amine, acid and alcohol moieties, and functional groups with higher hydrophilic character were produced; which resulted in the development of hydrophilic domains (bright regions). Thus, degradation may continue via additional water penetration. Consequently, hard segments or less hydrophilic domains with potential biodegradation may also be gradually involved and resulted in the development of hydrophilic domains and deterioration of hydrophobic domains (dark regions), as well.

Cell cytotoxicity

For initial cytotoxicity evaluation, L929 mouse fibroblast cells are well-proven cell lines, due to easy proliferation and adherence to the most of the biomaterial surface [39, 40]. L929 cells were used in this study because they can be easily cultured in a reproducible way. The PEU sample was incubated with monolayer cells at 37 °C for 24–48 h. The PEU surface was studied using an optical microscope for surveying the cellular response after the specific incubation time. Cell cytotoxicity of the PEU sample was compared with a negative control (a nontoxic material: fibroblast cell culture) and a positive control (a toxic substance, i.e., stabilized PVC disk). The cell monolayer was examined microscopically for a response around the test sample.

The cell density on the negative control (Fig. 5a) is very similar to the fibroblast-like morphology with cell-to-cell contacts and filopodia extension. In contrast, the L929 cells are almost dead on the positive control (stabilized PVC disk) revealing severe toxicity (Fig. 5b). Similar to the negative control, the cells in contact with the PEU showed spindle-shaped morphology characteristics of the mouse fibroblast cell line. The spindle-shaped L929 cells are found to adhere and expand on PEU (Fig. 5c). The cell adhesion studies after 48 h, show that the cells have excellent adherence to the surface of PEU and the cell proliferation on polymer surface continued (Fig. 5d).

Furthermore, to evaluate the proliferation of the cells neighboring the polymer particles, we carried out an extra test in which we examined the medium of cells culture 48 h after removing the significant parts of the polymer; it clearly illustrates that the L929 cells have been able to continue their proliferation (Fig. 5e). Thus, the PEU sample has no considerable toxicity on the L929 cell line. The results suggest that the PEUs samples, PA1 or PA2, may be considered as candidates for further biomedical studies.

Functionalization of MWCNTs

Recently, the attention to biomaterials containing nano-fillers is rapidly increasing. In this study, MWCNTs were used as nano-fillers for biodegradable nontoxic PEUs, and for the first time, we prepared their mixed water dispersion composites. However, the primary limitation to this end was de-bundling and accumulation of MWCNTs in the polymer matrix owing to their first intramolecular Van der Waals forces and hydrophobic interactions. It has been shown that generating functional groups on CNTs surfaces successfully facilitates their interaction with the polymer matrix. One of the most usual chemical modification methods of CNTs is their surface modification to form oxygen-containing functional groups. The total oxygen content of MWCNTs by Boehm titration method was 0.01 and 1.3 mmol/g before and

Fig. 5 L929 cells incubated with **a** negative control, **b** positive control, **c** PEU sample over 24 h, **d** PEU sample over 48 h, **e** the medium of cells culture after removing the major parts of polymer after 48 h

after treatment, respectively. In this work, due to the existence of AS moiety in the polymer structure, the MWCNTs were functionalized with aspartic acid (AS) as a bio-based molecule to improve interfacial interactions between the latter and former. Functionalization was done under microwave heating using a simple and low-cost method via formation

of amide linkages between COOHs of MWCNTs and NH_2 of AS (Scheme S5) [38, 39, 59].

PEU/MWCNT-AS composites

The PEUs containing different amounts of MWCNTs-AS were fabricated using the casting solution method (Scheme 1). The carboxylic acid moieties initially presented on the surface of MWCNTs can interact with the functional groups of PEUs through hydrogen bonding interactions.

Scheme 4 All different possible hydrogen bonding interactions between functional groups of PEUs chains and functional groups of MWCNT-AS (urethane NH $\cdots$ O=C aspartic acid on CNT or urethane HN $\cdots$ HO aspartic acid on CNT or urethane NH $\cdots$ O=C carboxylic acid on CNT or urethane HN $\cdots$ HO carboxylic acid on CNT or urethane C=O $\cdots$ HO aspartic acid on CNT or urethane C=O $\cdots$ HO carboxylic acid on CNT or polymer aspartic

acid OH $\cdots$ O=C aspartic acid on CNT or polymer aspartic acid C=O $\cdots$ HO aspartic acid on CNT or polymer aspartic acid OH $\cdots$ O=C carboxylic acid on CNT or polymer aspartic acid C=O $\cdots$ HO carboxylic acid on CNT or polymer aspartic acid HO $\cdots$ HO carboxylic acid on CNT) (This scheme DO NOT intend to show real polymer and CNT locations and interaction lay out. This only shows all different possible hydrogen bonding interactions)

Scheme 4 shows different possible hydrogen bonding interactions between functional groups of PEUs such as urethane NH, urethane C=O, aspartic acid OH and aspartic acid C=O, and functional groups of MWCNTs-AS such as aspartic acid OH, aspartic acid C=O and COOH groups of MWCNTs. These interactions can affect thermal, dispersion and degradation properties of the polymers as well as water dispersion and polymer dispersion characteristics of MWCNTs.

FTIR analysis of functionalized MWCNTs

FTIR analysis confirmed the functionalization of MWCNTs. The FTIR spectra of the MWCNTs-OX and MWCNT-AS are shown in Fig S2 (a and b spectra). The presence of the intense and broad absorption band centered at 3425 cm^{-1} , attributed to the O–H stretching bands of carboxylic acid (COOH) and hydroxyl (OH) moieties attached to the surface of MWCNTs confirms the oxidation of MWCNTs (Fig. S2, spectrum a). The C–H stretching band around

2919 cm^{-1} , the C=O stretching vibration of carboxylic acids at 1707 cm^{-1} and the C–O stretching band of hydroxyl groups at 1165 cm^{-1} verify the oxidation of MWCNTs.

The spectrum of MWCNT-AS shows the appearance of two absorption bands at 1634 and 3438 cm^{-1} assigned to the amide NH and C=O, respectively. The modified MWCNTs were treated with 5 wt% HCl solution to avoid aggregation and to get better dispersion. The presence of carboxylic acid and amidic C=O stretching vibrations at 1710 and 1634 cm^{-1} , respectively, confirmed the functionalization of MWCNTs with aspartic acid (Fig. S2, spectrum b).

Morphology of functionalized MWCNTs and PEU/MWCNTs-AS composites

The microstructures of the MWCNT-OX and MWCNT-AS were studied by FESEM (Fig. 6). FESEM micrographs of pure PA1 and PA1/MWCNT-AS composite are shown the morphology and dispersity of MWCNTs-AS in the polymer matrix. The pure PEU showed spherical particles which

Fig. 6 FE-SEM micrographs of the surface morphology of pure PEU (PA1) (a), PEU/MWCNT-AS (b), MWCNT-TOX (c) and MWCNT-AS (d)

were self-arranged in nanoscale size (Fig. 6a). The average diameter of polymeric particles was about 300 nm. PEU/MWCNT-AS composite showed well dispersed and embedded nano-fillers in PEU matrix (Fig. 6b) where, particle size had reduced sharply and MWCNTs-OX showed a smooth surface (Fig. 6c). The surface of MWCNT-AS possessed an uneven and de-bundled structure (Fig. 6d).

Comparison of the FESEM micrographs of pure PEU and PEU/MWCNT-AS composite (Fig. 6a, b) illustrated that the morphology of the polymer was changed after adding MWCNT-AS. According to FESEM micrograph (Fig. 6b), there are no MWCNTs migrated on the surface of

Fig. 7 TEM micrographs of PA1/MWCNT-AS (2 wt%) composite sample

Fig. 8 TGA thermograms of MWCNT-OX, MWCNT-AS, pure PEU (PA1) and PA1/MWCNT-AS composite (2 wt%)

Table 3 TGA data of MWCNT-OX, MWCNT-As, pure PEU (PA1) and PA1/MWCNT-AS (2 wt%) samples

Specimen	T_i (°C) ^a	$T_{5\%}$ (°C) ^b	$T_{10\%}$ (°C) ^c	Char residue ^d (%)	LOI (%) ^e	ΔH_{comb} (J/g) ^f
MWCT-OX	300	500	> 800	> 90	53.5	149.5
MWCNT-AS	227	300	395	60	41.5	192.7
PEU (PA1)	160	220	253	5	19.5	410.3
PA1/MWCNT-AS	226	306	340	26	27.9	286.7

^aTemperature at which initial weight loss was started at a heating rate of 10 °C/min in nitrogen atmosphere^bTemperature at which 5% weight loss was recorded at a heating rate of 10 °C/min in nitrogen atmosphere^cTemperature at which 10% weight loss was recorded at a heating rate of 10 °C/min in a nitrogen atmosphere^dPercentage weight of residue of material which left undecomposed after TGA analysis at maximum temperature 800 °C in nitrogen atmosphere^eLimiting oxygen index (LOI) calculated at char residue at 800 °C^fHeat of combustion calculated from LOI

the polymer; however, there are several holes and needle-like structures on the polymer surface that can show the existence of MWCNT-AS in the polymer matrix and also the wrapping of MWCNTs-AS by polymer chains through different ways of interactions between functional groups of modified MWCNTs and PEU chains. It is clear that covalent bonding, Van der Waals interactions and hydrogen bonding between COOH, OH, NH and C=O groups on the surface and edges of MWCNTs-AS, and the amide, urethane, urea and acid functional groups of polymer chains were responsible for these phenomena. Furthermore, π – π interactions between MWCNTs-AS caused more connections. all of these factors caused the polymer to wrap around MWCNTs and also made changes in the polymer morphology (Scheme 4).

Figure 7 shows the TEM image of the functionalized MWCNTs dispersed in the PA1 matrix. It can be seen that the interfacial interactions between the MWCNT-AS and the PEU matrix play an important role in improving the dispersion of MWCNTs in the PEU matrix and the polymer composite properties. The interactions between several organic functional groups especially OH, NH, and C=O groups, lead to the better interaction of the PEU chains with MWCNT-AS and mutually better dispersion of MWCNTs-AS in the PA1 matrix.

TGA

Figure 8 shows the TGA curves of pure PA1, modified MWCNTs samples and PA1/MWCNT-AS composite. The data obtained from these studies are presented in Table 3. The major single step degradation was observed for all the samples.

The TGA curve of oxidized CNTs showed a small mass loss starting at 300 °C. The weight loss of MWCNTs functionalized with AS started at 227 °C resulted from the

decomposition of the organic functional groups attached to the MWCNTs.

TGA data of the modified MWCNTs suggests that the MWCNTs can resist in thermo-degradation temperatures up to 800 °C. The temperature at which 5% weight loss was recorded for MWCNTs-OX was about 500 °C, indicating that the MWCNTs-COOH were thermally stable up to this temperature, while the AS functionalized MWCNTs slowly lost 5% of their weight at about 300 °C owing to losing organic functional groups. Thus, it can be concluded that the thermal stability of MWCNTs-OX has reduced after its functionalization with AS.

Comparing TGA curves of pure PEU with PEU/MWCNT-AS composite, it can be seen that the thermal stability of the PEU/MWCNT-AS composite has been increased. This can be due to the higher thermal stability and thermal conductivity of MWCNTs that facilitate heat dissipation within the polymer nanocomposites. Therefore, this thermal conductivity prevents the accumulation of heat at specific points within the polymer matrix for degradation. This property can induce sterilization ability of the polymer for medical purposes. The weight residue after significant degradation at 800 °C for PEU/MWCNT-AS composite was higher than that of pure PEU as well. Limiting oxygen indexes (LOI) of PEU and PEU/MWCNT-AS composite and their heat of combustion were calculated upon their respective char residues at 800 °C according to Eqs. (3) and (4). It is said that a material with $21\% < \text{LOI} < 28\%$ is considered as a “slow-burning” material. A material with LOI higher than 28% would be considered as a “self-extinguishing” material. Thus, PEU and PEU/MWCNT-AS composite can be considered as relatively “slow-burning” materials.

According to Van Krevelen [60] and Johnson [61] for halogen-free polymers, two theoretical relationships exist between the LOI and the parameters of the combustion process: char residue (CR) and heat of combustion (ΔH_{comb}).

Table 4 Particle size and zeta potential data of dispersed composite PEUs/MWCNT-AS-D

Dispersed composite	Potential (mV) ^a	Z-avg (nm) ^d
PA1/MWCNT-AS-D	-22.5 ± 0.1^b , -18.5 ± 0.1^c	200 ± 0.1^e , 100 ± 0.12^f , 50 ± 0.11^g
PA2/MWCNT-AS-D	-23.82 ± 0.1^b , -18.8 ± 0.1^c	320 ± 0.22^e , 160 ± 0.14^f , 85 ± 0.21^g

^aZeta potential of dispersed composite (with 0.5^b and 2^c wt% MWCNT-As percentage content; ^dthe average of particle size of dispersed composite (with 0.5^e, 1^f, and 2^g wt% MWCNT-As percentage content)

From these equations, the higher the char residue of a material, the better will be its flame retardant property.

$$\text{LOI} = 17.5 + 0.4 \text{ CR}, \quad (3)$$

$$\text{LOI} = 8000 / \Delta H_{\text{comb}}, \quad (4)$$

where ΔH_{comb} is the specific heat of combustion in J/g.

PA1 showed a minor thermal degradation of about 13% weight loss between 160 and 293 °C in step 1. It showed its major thermal degradation of about 68% between 293 and 513 °C and immediately after that it showed another 13% weight loss between 513 and 800 °C. The PA1/MWCNT-AS composite showed its major thermal degradation of about 63% between 226 and 566 °C and immediately after that it showed another 11% weight loss between 566 and 800 °C.

Dispersion properties of PEU/MWCNT-AS-D

We analyzed the particle sizes of dispersed composites, PA1/MWCNT-AS-D and PA2/MWCNT-AS-D, with different contents of MWCNT-AS by the Zetasizer instrument. The results show that the particle sizes of dispersed composites in water (pH 8) were in the range of 50–200 nm for PA1/MWCNT-AS-Ds and the particle sizes of dispersed PA2/MWCNT-AS-Ds were in the range of 85–320 nm (Table 4).

In addition, the zeta potentials of dispersed composite samples were measured in this study. Zeta potentials were calculated based on the Eq. (2). The results are reported in Table 4 and found to be -22.5 mV for PA1/MWCNT-AS(0.5%)-D and -23.82 mV for PA2/MWCNT-AS (0.5%)-D. The negatively charged particles do not agglomerate during particles dispersion.

As previously mentioned, the stability of a dispersed system depends on its level of zeta potential, molecular weight and particle size distribution. It can be seen that the particle sizes of composites have decreased with increasing the nano-filler content in comparison with the particle size of the pure PEU dispersion. On the other hand, the zeta potentials of the dispersed particles with increasing filler content have decreased. As mentioned before, the surface potential will increase with increasing the size of particles. However, despite decrease in the surface potentials of the dispersed particles compared with that of pure PEU, they have longer dispersion stabilities (for more than 100 days); this is because of their higher ratios of surface zeta potentials

to smaller particle size (higher charge density) and the presence of more acidic groups on their surface compared with pure PEU (Scheme S6).

Degradation of PEUs/ MWCNT-AS composites in PBS solution

The hydrolytic degradations of PA1/ MWCNT-AS (2%) and PA2/ MWCNT-AS (2%) polymer composites were studied in PBS solution (pH 7.4) at 37 °C and were compared with those of PA1 and PA2. The results showed that the percentages of weight losses of the two composites samples were lesser than those of PA1 and PA2, respectively. The weight loss percentages of PA1/MWCNT-AS (2%) and PA2/MWCNT-AS (2%) were 19% and 39.5%, respectively. It can be said that the inclusion of carbon nano-fillers into the PEU matrix, due to their strong SP2 carbon–carbon bonding and their geometric arrangement, has enhanced the strength of the polymer matrix. Lower degradation of composite samples compared with pure polymer samples can be due to more interchain interactions of the composites relative to pure polymers. Due to the interaction of several functional groups of the PEU chains with functional groups of MWCNTs-AS, it can be expected that the chain packing distances of the polymer on MWCNTs decreases, leading to better interactions of the PEU chains with MWCNTs-AS. These compacted interactions between PEU chains and MWCNTs-AS decreased the insertion of water molecules into the PEU–PEU and PEU–MWCNT-AS chains spaces (Scheme S7).

In addition, water uptake study of polymers and composites showed that composites incorporating MWCNTs, are more hydrophobic compare with the polymers. Thus, it can be said that hydrophobic effect of MWCNTs can play a role on reduction of degradation of PEUs/MWCNT-AS composites. The maximum water uptake of PA1, PA2 and PA1/MWCNT-AS were occurred after 11, 13 and 18 h, respectively. Their maximum water-uptakes were 600, 775 and 437%, respectively. Their equilibrium water uptakes after 72 h were 387, 437 and 225%, respectively (Fig S3).

Conclusion

PUs have good physicochemical properties, which make them appropriate candidates for biomedical applications especially in drug delivery systems. Moreover, to make PUs as bio-friendly systems, designing water based PUs with bio-friendly monomers can be a promising strategy. Embedding functionalized CNTs (f-CNT) into polymer matrices which leads to a good distribution of f-CNTs is expected to develop new potential applications in diverse fields. Recently, CNTs have been raised as excellent nano-carriers for the highly efficient delivery of drugs and biomolecules. Thus, in this study for the first time, two different newly designed water-dispersed polymers and dispersed CNT nano-composites were prepared. The dispersed nano-composites were prepared via the simultaneous dispersion of functionalized MWCNTs and new PEUs. The PEUs based on PEG-1000 and amino acids were successfully synthesized by the reaction of NCO-terminated oligo-ether urethanes consisting of HDI-PEG as hydrophilic soft segments with LAC as the peptide-based chain extender. Hydrophobic hard segments were originated from the reaction of an amino acid-based monomer, LAC, with HDI. The polymerization reactions were performed in NMP as a solvent in the absence of any catalyst. Notably in this work Aspartic acid (AS) was successfully used for end functionalization of polymer chains and MWCNTs' surfaces as a bio-friendly dispersing agent.

Our comprehensive studies show that synthesis methods, the sequence of addition of monomers and monomers ratios have influences on the polymer chain structures, surface zeta potentials, water penetration into particles and degradation rates, particle sizes and hydrophilic/hydrophobic segments distributions along the polymer chains. Thus, it is possible to design completely different shaped polymeric scaffolds with different degradation and dispersion properties. The degradation study of polymers in PBS solution suggested the idea that the degradation is a hydrolysis process and after 10 days of immersion, the weight losses were about 23.24% (PA1) and 44.44% (PA2). PA1 and PA2 showed no toxicity with L929 fibroblast cell line. In addition, in this study, functionalized MWCNTs-AS under microwave irradiation were prepared as fast and economic route. Then polymer/MWCNT-AS composites and polymer/MWCNT-AS water dispersion composites were manufactured. Interestingly, it was shown that the degradation rate of polymers and the size of water-dispersed-composites aggregates decreased with increasing MWCNT-AS loading. It can be seen that PEU/MWCNT-AS composites show improved dispersion of MWCNTs-AS in the PEU matrix, increased thermal stability and enhanced water dispersion stabilities for more than 100 days. It can be said that the inclusion of carbon nano-fillers into PEU matrix has enhanced the strength of the polymer matrix,

owing to the interactions of several functional groups of the PEU chains with functional groups of MWCNTs-AS. This improved feature has reduced degradation of each composite sample compared with pure polymer samples. Compacted interaction between PEU chains and MWCNT-AS as well as hydrophobic effect of CNTs decreased the insertion of water molecules into the PEU-PEU and PEU-MWCNT-AS chains spaces which results in lower degradation rates and reduced particle sizes.

This study showed that MWCNTs-AS have affected thermal, degradation and dispersion properties of PEUs. Furthermore, interaction of polymer chains on the surface of MWCNTs improved water disperse-ability of CNTs. Our primary studies showed that these dispersed polymers and their PEU/MWCNT-As composites dispersions show tunable properties such as surface zeta potentials, water absorptions, degradation rates and particle sizes which make them appropriate candidates as potential drug carriers.

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