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Evaluation of biodegradability on polyaspartamide-polylactic acid based nanoparticles by chemical hydrolysis studies

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ABSTRACT

Here, the synthesis of two graft copolymers based on α,β-poly(N-2-hydroxyethyl)-D,L-aspartamide (PHEA) and poly(lactic acid) (PLA), the O-(2-aminoethyl)-O′-galactosyl polyethylene glycol (GAL-PEG-NH_2) or the methoxypolyethylene glycol amine (H_2N-PEG-OCH_3) is described. Starting from the obtained PHEA-PLA-PEG-GAL and PHEA-PLA-PEG copolymers, polymeric nanoparticles were prepared by high pressure homogenization–solvent evaporation method.

To demonstrate their biodegradability as a function of the matrix composition, a chemical stability study was carried out until 21 days by incubating systems in two media mimicking physiological compartments (pH 7.4 and pH 5.5). The degradability of both nanosystems was firstly confirmed by the reduction of the pH of the incubation medium. Moreover, the percentage yield of recovered nanoparticles show a gradual reduction while mean size increases as a function of incubation time. Degradation seems to be mainly attributed to the loss of water-soluble portions of PLA, and proceeds with greater speed at pH 5.5, than at pH 7.4 and as a function of matrix composition.

1. Introduction

Nanomedicines today represent the most promising tools to manage diseases such as cancer, AIDS, and neurodegenerative disorders, so that some of them are currently tested in clinical trial phases and some under marketing [1–3]. Among advantages of nanomedicines towards conventional formulations, nanoencapsulation of therapeutically active compounds increases drug efficacy and tolerability, bioavailability, retention time and improves intracellular penetration [3].

To obtain increasingly effective nanomedicines, a great effort has been done in order to obtain biocompatible and biodegradable polymers that: (1) not cause any inflammatory response; (2) control the release of drugs into specific sites in the body; (3) possess a degradation time suitable with their function; (4) show appropriate mechanical properties, permeability and processability for the designed application; and (5) produce nontoxic degradation products that can be readily resorbed or excreted from the body by natural metabolic pathways, thus giving minimal side effects [4,5]. These properties are greatly affected by a number of their features such as chemical composition, molecular weight, hydrophobicity, surface charge, water adsorption, and degradation mechanism. Thus, understanding the degradation processes of biomaterials is of critical importance for their use in nanomedicine [4].

Poly(lactic acid) (PLA) is biodegradable, bioresorbable, and biocompatible polymer with well-established properties suitable for the realization of polymeric nanoparticles for drug delivery and targeting, being PLA FDA-approved for biomedical applications [3]. However, the polymeric crystallinity of PLA could interfere with controlled degradation [6–8]. Thus, to obtain nanoparticles with tunable properties such as size, surface charge and hydrophilicity, and degradation rate, novel PLA-based copolymers were synthesized by following block or graft copolymerization reactions with other polymers, such as other polyesters, chitosan (CS), polyethylene glycols (PEGs), and fatty acids [9–12].

Considering the importance of nanomedicines and the use of biocompatible and biodegradable polymers with tunable properties for their preparation, in this paper, the synthesis of two novel graft copolymers based on α,β-poly(N-2-hydroxyethyl)-DL-aspartamide (PHEA) and PLA is reported [13,14].

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PHEA is a biocompatible protein-like copolymer, whose successful use as starting material for several biomedical and pharmaceutical applications has been reported in previous papers [15–22].

PHEA was functionalized with PLA to obtain the amphiphilic graft copolymer PHEA-PLA. The latter was subsequently functionalized with PEG or a PEG derivative containing galactose (GAL) molecules at the end chain, in order to obtain PHEA-PLA-PEG or PHEA-PLA-PEG-GAL copolymers, respectively. The resulting two graft copolymers were properly characterized by ^{1}H NMR, FT-IR and SEC analyses, and subsequently used for the preparation of nanoparticles by using the high pressure homogenization-solvent evaporation technique. Nanoparticles were easily obtained without the use of surfactants or stabilizing agents, and then properly characterized in terms of mean size, zeta potential and morphology.

In order to investigate the biodegradability of obtained particles as a function of the chemical composition and incubation time, a chemical degradation study was carried out on both PHEA-PLA-PEG and PHEA-PLA-PEG-GAL nanoparticles. In particular, each nanoparticle system was incubated at 37 °C in isotonic media mimicking physiological conditions (pH 7.4 and pH 5.5). At increasing incubation times until 21 days, the pH of the incubation medium was measured and then, after proper purification of the nanoparticle dispersion, on the recovered product ^{1}H NMR, FT-IR, PCS and SEC analyses were carried out in order to obtain qualitative and quantitative information on the hydrolysis process undergone by nanoparticles.

2. Materials and methods

2.1. Materials

Anhydrous N,N′-dimethylformamide (a-DMF), D,L-poly(lactic acid) (PLA acid terminated, 10–18 kDa), α-lactose, sodium cyanoborohydride, 1,1′-carbonyldiimidazole (CDI), anthrone, anhydrous dimethylacetamide (a-DMA), disuccinimidyl carbonate (DSC) Poly(ethylene oxide) standards, were purchased from Sigma–Aldrich (Italy). Diethylamine (DEA), triethylamine (TEA), O-(2-Aminoethyl)-O′-methyl poly(ethylene glycol) 2000 (PEG_{2000}) (≤0.4 mmol NH_2/g), Poly(ethylene glycol) bis(amine) 2000 (H_2N-PEG-NH_2), ethyl ether, dichloromethane were obtained from Fluka (Italy). All reagents were of analytic grade, unless otherwise stated.

α,β-Poly (N-2-hydroxyethyl)-D,L-aspartamide (PHEA) was prepared by aminolysis of polysuccinimide (PSI) with ethanolamine in DMF solution [13]. Spectroscopic data were in agreement with the attributed structure. ^{1}H NMR (300 MHz, D_2O, δ): 2.8 (m, 2H, –CH**CH_2**CONH–), 3.3 (m, 2H, –NH**CH_2**CH_2OH), 3.6 (m, 2H, –NHCH_2**CH_2**OH), 4.7 (m, 1H, –NH**CH**(CO)CH_2–). Weight average molecular weight ($\overline{M}_w$) of PHEA, determined by size exclusion chromatography (SEC) analysis, was 38.7 KDa ($\overline{M}_w/\overline{M}_n = 1.62$).

SEC system (Waters, Mildford, MA) was equipped with a pump system, two Phenogel columns from Phenomenex (5 μm particle size, 10^3 Å and 10^4 Å of pores size), and a 410 differential refractometer (DRI) as concentration detector. Analyses were performed with 0.01 M LiBr DMF solution as eluent with a flow of 0.8 ml/min and poly(ethylene oxide) standards (range 145–1.5 kDa) to obtain the calibration curve. The column temperature was set at 50 °C (±0.1 °C).

2.2. Synthesis of PHEA-PLA graft copolymer

Derivatization of PHEA with acid terminated PLA to obtain the PHEA-PLA graft copolymer was carried out by using CDI as coupling agent to activate the terminal carboxyl group of PLA by using a before reported procedure [23].

Briefly, a proper amount of CDI dissolved in a-DMF was added to a PLA solution in the same solvent; after 4 h of activation at 40 °C, the PLA activated solution was added to a PHEA solution in the presence of DEA as catalyst [23]. The reaction was left under argon atmosphere and continuous stirring at 40 °C for 70 h, then precipitated in diethyl ether:dichloromethane mixture (15:1 vol/vol), and the obtained suspension was centrifuged. Then, obtained white product was carefully dried under vacuum. PHEA-PLA copolymer was obtained with a yield of 305 wt% based on the starting PHEA.

2.3. Characterization of PHEA-PLA graft copolymer

PHEA-PLA derivative was characterized by ^{1}H NMR and SEC analyses.

^{1}H NMR spectra were obtained by a Bruker Avance II-300 spectrometer, working at 300 MHz.

^{1}H NMR (300 MHz, DMF-d_7, δ): 1.3 and 1.7 (2d, 582 H_{PLA} –O–CO–CH(**CH_3**)–O–); 2.8 (m, 2H_{PHEA} –CO–CH–**CH_2**–CO–NH–); 3.3 (t, 2H_{PHEA} –NH–**CH_2**–CH_2–O–); 3.6 (t, 2H_{PHEA} –NH–CH_2–**CH_2**–O–); 4.2–4.5 and 5.1–5.5 (m, 194 H_{PLA} –O–CO–**CH**(CH_3)–), 4.8 (m, 1H_{PHEA} –NH–**CH**(CO)CH_2–).

$\overline{M}_w$ of PHEA-PLA graft copolymer was found to be 93.4 kDa ($\overline{M}_w/\overline{M}_n = 1.83$).

2.4. Conjugation of lactose onto poly(ethylene glycol) bis(amine) (H_2N-PEG-NH_2)

The O-(2-aminoethyl)-O′-galactosyl polyethylene glycol (H_2N-PEG-GAL) derivative was synthesized by a reductive amination reaction [24,25]. Briefly, H_2N-PEG-NH_2 (800 mg, 0.4 mmol) was dissolved in borate buffer at pH 9 (16 mL) at 40 °C, and lactose (85.6 mg, 0.25 mmol) and sodium cyanoborohydride (15.7 mg, 0.25 mmol), dissolved in the same medium (2 mL), were added determined according to $R_1 = 2.5$, where:

$$R_1 = \frac{\text{moles of lactose}}{\text{moles of free} - NH_2 \text{ on } H_2N - PEG - NH_2} \quad (1)$$

and $R_2 = 1$, where

$$R_2 = \frac{\text{moles of lactose}}{\text{moles of sodium cyanoborohydride}} \quad (2)$$

The obtained reaction mixture was left to react for 24 h at 40 °C, then was dialysed (Spectra/Por® Standard RC tubing; MWCO 1 kDa) against distilled water for 24 h and lyophilised.

2.5. Characterization of H_2N-PEG-GAL derivative

The content of amine-terminated side chains was determined by modified TNBSA assay [26]. H_2N-PEG-GAL (10 mg) was solubilised in borate buffer at pH 9.3 (1 ml). Twenty-five μl of this solution were added to 950 μl of borate buffer and 25 μl of 0.03 M TNBSA solution. After 90 min incubation, absorbance at λ = 500 nm was measured by recording UV spectra by a RF-5301PC spectrofluorometer (Shimadzu, Italy). Values were compared with a calibration curve obtained with H_2N-PEG-NH_2 (–NH_2 in the range between 0.01 and 0.001 mmol ml^{-1}).

The anthrone-sulfuric acid colorimetric assay was used to determine the content of GAL in H_2N-PEG-GAL derivative [25,27].

Anthrone-sulfuric acid reagent was prepared before use, and then 1.5 ml of this solution was added to 0.5 ml of H_2N-PEG-GAL