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Fast *in vitro* hydrolytic degradation of polyester urethane acrylate biomaterials: Structure elucidation, separation and quantification of degradation products

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ABSTRACT

Synthetic biomaterials have evoked extensive interest for applications in the field of health care. Prior to administration to the body a quantitative study is necessary to evaluate their composition. An *in vitro* method was developed for the quick hydrolytic degradation of poly-2-hydroxyethyl methacrylate (pHEMA), poly(lactide-co-glycolide50/50)1550-diol (PLGA(50:50)$_{1550}$-diol), PLGA(50:50)$_{1550}$-diol(HEMA)$_2$ and PLGA(50:50)$_{1550}$-diol(etLDI-HEMA)$_2$ containing ethyl ester lysine diisocyanate (etLDI) linkers using a microwave instrument. Hydrolysis time and temperature were optimized while monitoring the degree of hydrolysis by ^{1}H NMR spectroscopy. Complete hydrolytic degradation was achieved at 120 °C and 3 bar pressure after 24 h. Chemical structure elucidations of the degradation products were carried out using ^{1}H and ^{13}C NMR spectroscopy. The molecular weight (MW) of the polymethacrylic backbone was estimated via size-exclusion chromatography coupled to refractive index detection (SEC-dRI). A bimodal MW distribution was found experimentally, also in the pHEMA starting material. The number average molecular weights (M_n) of the PLGA-links (PLGA(50:50)$_{1550}$-diol) were calculated by high pressure liquid chromatography–time-of-flight mass spectrometry (HPLC–TOF-MS) and ^{1}H NMR. The amounts of the high and low MW degradation products were determined by SEC-dRI and, HPLC–TOF-MS, respectively. The main hydrolysis products poly (methacrylic acid) (PMAA), ethylene glycol (EG), diethylene glycol (DEG), lactic acid (LA), glycolic acid (GA) and lysine were recovered almost quantitatively. The current method leads to the complete hydrolytic degradation of these materials and will be helpful to study the degradation behavior of these novel cross-linked polymeric biomaterials.

1. Introduction

Synthetic polymeric biomaterials are of high importance in the medical field due to an aging population and their potential to improve the quality of life [1]. There is a gradual trend to replace non-degradable materials with degradable materials mainly because of the need to avoid reinterventions when complications arise with non-degradable materials [2]. This is most vividly seen with the move in the stent coating area where stable drug eluting coatings are being replaced with biodegradable coatings [3]. Such kinds of materials have their potential use as joint and limb replacements [4], artificial arteries [5] and skin [6], contact lenses [7], dental implants [8], catheters [9], in tissue engineering [10] and as systems for controlled delivery of drugs [11], etc. An important class of degradable biomaterials are chemically cross-linked polymeric networks predominantly based on pHEMA and PLGA [12,13]. Since its birth in 1936 [14] and first reported application for contact lenses in 1960 [15], pHEMA is one of the most extensively studied polymeric biomaterials in biomedical applications [16] because of its biocompatibility, hydrophilicity, softness, high water content and permeability [17], but it has poor mechanical properties [18]. However, numerous studies reported the modification of the hydroxyl group with poly(ε-caprolactone) (PCL) [3], poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [19], dextran [11], poly(2-(dimethylamino)ethyl methacrylate) [20], poly(ethylene oxide) [12], poly(tetrahydrofurfuryl methacrylate) [21], poly(ethylene

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glycol)-methacrylate [22], poly(dimethylsiloxane) [23], sulfopropyl methacrylate [24] and cross-linker to tune the biomechanical properties of the pHEMA.

PLGA is an FDA-approved biodegradable and biocompatible polymeric biomaterial [25]. PLGA is widely used as a drug delivery matrix using numerous forms such as microspheres [26], nanoparticles [27], scaffold [28], microfibers [29], tablets [30], in the field of control release delivery devices, and tissue engineering. Currently, the focus on synthesis of copolymers of PLGA with other polymers has been increased such as PLGA-PCL-PLGA [31], MeO-PEG-PLGA-PEG-OMe [32], PLGA-PEG [33] and PLGA-grafted dextran [34].

Chemical and enzymatic hydrolysis are the primary biodegradation mechanisms for such materials. Phagocyte-derived oxidants, produced as a result of foreign body response, may also contribute to the *in vivo* degradation of aliphatic ether groups in these networks [35]. The suitability of the polymeric biomaterials for medical devices can be inferred from their chemical structure, the degradation time and the biocompatibility of the polymers and their degradation products [11]. Swelling ratios (water contents) of the hydrogels [10,12], weight loss [10,23], pH of the medium [36], kinetic chain length [37], etc. are the most common parameters used to assess the *in vitro* degradation of material. These parameters may be insensitive in the early stages of degradation and are not very informative on toxicology. Chromatographic methods that can give more insight into the structure of these networks and can be used to predict their properties more accurately are desired. However, networks lack solubility, a prerequisite for such analysis. This requires a very sensitive method of analysis, or at least an accelerated *in vitro* chemical hydrolysis of the novel biomaterials at extreme pHs or high temperature, possibly avoiding the formation of any insoluble product, followed by the structural analysis and quantification of their degradation products. The collected information will be helpful not only (i) to ascertain the composition of the original networks, but also (ii) to evaluate the biocompatibility of these polymeric networks and their degradation products and (iii) to modify the existing and to design new biomaterials for specific applications. Recently, Matsubara et al. reported a supercritical methanolysis to achieve the selective decomposition at ester linkages in a UV-cured acrylic ester resin to characterize the cross-linking structures, but no quantification of the decomposition products was done to assess the degree of methanolysis [38].

A more detailed second approach to study these prospective biomaterials is a chemical or a specific enzymatic degradation during physiological conditions, allowing one to study the kinetics of degradation. Again, specific and sensitive chromatographic methods will be needed to draw sound conclusions. In particular a method is needed as the second stage in a two-step procedure and is reported here. First degradation under physiologically relevant conditions is performed, resulting in partially degraded material of which the constituents may be identified. Then complete and fast degradation of the products of the first step (oligomers, intermediates and other products) is executed for quantification.

In the present study polymeric biomaterials based on pHEMA (backbone) and PLGA(50:50)$_{1550}$-diol (PLGA-links) were subjected to fast hydrolytic degradation. One reason to select these samples is that pHEMA, frequently formed as an intermediate hydrolysis product in polymeric network biomaterials is only partially hydrolyzed under physiologically relevant conditions [11] and no detailed study on the complete hydrolytic degradation and direct analysis of its degradation products has yet been published to our knowledge.

In this paper first the development and optimization of a method for the microwave-assisted *in vitro* hydrolytic degradation is reported of pHEMA, PLGA(50:50)$_{1550}$-diol and the photo-crosslinked polymeric biomaterials such as PLGA(50:50)$_{1550}$(HEMA)$_2$ and PLGA(50:50)$_{1550}$-diol(etLDI-HEMA)$_2$. The hydrolysis of polymeric biomaterials and their model building blocks, pHEMA (backbone) and PLGA(50:50)$_{1550}$-diol (PLGA-links) were performed at up to 120 °C, for different periods of time. The hydrolysis time and the temperature were optimized while monitoring the degree of hydrolysis in the starting material with ^{1}H NMR spectroscopy. Then the structure elucidations of the degradation products (Fig. 1) were carried out using ^{1}H and ^{13}C NMR spectroscopy and quantification of high MW hydrolyzed polymethacrylic acid backbone by SEC-dRI and LA, GA, EG, DEG, and lysine by HPLC–TOF-MS in the hydrolyzed sample are reported. The MW distribution of the hydrolyzed backbone was estimated via SEC-dRI. The Mn of the PLGA-links was measured by HPLC–TOF-MS and ^{1}H NMR.

2. Experimental

2.1. Materials

DL-Lactide and glycolide were purchased from PURAC (CSM Biochemicals, Gorinchem, The Netherlands), ethyl ester of lysine diisocyanate from Kyowa Hakko Europe GmbH Dusseldorf, Germany), caprolactone from Solvay, methacryloyl chloride via Fluka. Irganox 1035 was obtained from Ciba-Geigy (Basel, Switzerland). pHEMA [M_v = 300 kDa (192,066) or 20 kDa (529,265), solvent and temperature conditions of M_v determination are not known] and all other chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA). The chemicals were used as such unless otherwise stated. All water used was deionized.

The experimental batches of PLGA(50:50)$_{1550}$-diol, PLGA(50:50)$_{1550}$-diol(HEMA)$_2$ and PLGA(50:50)$_{1550}$-diol(etLDI-HEMA)$_2$ were synthesized at DSM Biomedical, Geleen, The Netherlands, according to the following procedure:

Preparation of PLGA(50:50)$_{1550}$-diol: DL-lactide (51.6 g, 0.358 mol), glycolide (41.5 g, 0.358 mmol) and diethyleneglycol (6.85 g, 6.45 mmol) were weighed in the glovebox and melted at 150 °C under nitrogen conditions. 1 mL of a stock solution Tin(II)-ethylhexanoate (290 mg in 10 mL *n*-hexane) was added as a catalyst. The reaction was allowed to proceed for 18 h upon which the reaction mixture was cooled to room temperature to obtain poly(lactide-co-glycolide50/50)1550-diol [39].

Preparation of PLGA(50:50)$_{1550}$-diol(HEMA)$_2$: poly(lactide-co-glycolide50/50)1550-diol (100 g, 65 mmol), 200 mg Irganox 1035 and triethyl amine (13.05 g, 0.129 mol) were dissolved in 150 mL dry tetrahydrofuran. Methacryloylchloride (13.49 g, 0.129 mol) was added drop wise to the solution at controlled temperature (<5 °C). Immediately a white precipitate was visible (triethylamine.HCl salt). The dropping funnel was rinsed with THF (50 mL). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture cooled till 5 °C and filtered to remove the triethylamine.HCl salt. The THF was removed via evaporation with a rotavapor. The remainder was dissolved in 200 mL ethyl acetate. The clear solution was extracted once with 300 mL 0.1 HCl solution, once with 300 mL 5% NaCl-solution and 300 mL water. The resulting solution was dried with $NaSO_4$ and evaporated to dryness. poly(lactide-co-glycolide50/50)1550-dimethacrylate was obtained as a slightly coloured yellow oil. 30.49 g poly(lactide-co-glycolide50/50)1550-dimethacrylate, 13.1 g HEMA and 0.86 g Darocur 1173 was mixed in a clear formulation [39].

Microparticles preparation of PLGA(50:50)$_{1550}$-diol(HEMA)$_2$: 10.52 g of this formulation was mixed with 39.88 g PEG 35k (40% m/m in water), 30.0 g water and 5 g aceton. This mixture was stirred mechanically for 10 min at 800 rpm before polymerization. The polymerization was allowed to proceed for 60 min under UV light (Macam Flexicure controller, D-bulb, 200 mW/s/cm^2, Livingston, United Kingdom). After polymerization, the micro particles