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Degradation Behavior of an Amino Acid-Substituted Polyphosphazene with Triethyl Citrate as Plasticizer

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Abstract: A novel phenylalanine/ valine ethyl ester co-substituted polyorganophosphazene, incorporating triethyl citrate as plasticizer, is being investigated for its degradability. Films were solvent casted, and allowed to degrade for 4 weeks at an elevated temperature of 50 °C in water and two HEPES buffered solutions with pH values 6.8 and 7.4, respectively. The degradation of the samples in pure water occurred at a significantly accelerated rate compared to the samples incubated in HEPES buffer. Furthermore, based on their influence on degradation, the pH values of the media used were assessed.

Keywords: amino acid, polyorganophosphazene, polyphosphazene degradation, living cationic polymerization

1 Introduction

Biomaterial research is crucial for advancing healthcare by developing materials that can interact with biological systems in effective ways. The high demands placed on their mechanical properties, biocompatibility and non-toxic degradation products have led to great interest in novel polymers for several decades [1,2]. Polyphosphazenes are a class of polymers with great potential due to their wide range of properties. They consist of alternating phosphorus-nitrogen double bonds. By substituting the chlorine atoms of polydichlorophosphazenes, a wide variety of side groups are available, enabling the production of polymers with specific properties [3]. Therefore, various polyphosphazenes with different side groups have already been described in the literature [3]. Due to their biocompatibility, amino acid-substituted polyorganophosphazenes in particular offer an excellent material as implant coating and drug release system [4,5]. In this degradation study, the degradability of a novel amino acid-substituted polyorganophosphazene with triethyl citrate (TEC) as plasticizer in three different media is presented.

2 Material and Methods

Ph_3PCl_2, CH_3Cl, 2-(4-(2-hydroxyethyl)-1-piperazinyl)-ethanesulfonic acid (HEPES) and TEC were used as received. $Cl_3PNSiMe_3$ was synthesized according to literature known procedure [6]. All other chemicals were purified and dried by using commonly known purification and drying methods.
For one litre of 1 M HEPES buffer, 238.3 g of HEPES were dissolved in 800 ml water, followed by the dropwise addition of 1 M NaOH until the desired pH value (6.8 or 7.4) was reached. The solution was then filled up to 1 L.

2.1 Synthesis of Phenylalanine –/ Valine Ethyl Ester Co-Substituted Polyorganophosphazene

The synthesis was carried out under Schlenk conditions, using the well described living cationic polymerization with Ph_3PCl_2 (1 eq., 21 mg, 0.0598 mmol) as initiator and $Cl_3PNSiMe_3$ (622 eq., 8.37 g, 37.28 mmol) as monomer [7], to yield a polydichlorophosphazene ($PPZ^{Cl/Cl}$) with roughly $n = 600$ repeating units. Complete polymerization was indicated after 48 h of stirring at 25 °C by ^1H and ^{31}P NMR spectra of an aliquot of the reaction mixture. No signal of the monomer but

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a signal of the polydichlorophosphazene ($PPZ^{Cl/Cl}$) at −17.9 ppm in ^{31}P NMR was observed [8]. The reaction mixture was dissolved in CH_2Cl_2 and transferred to a white suspension of valine ethyl ester hydrochloride (8.56 g, 37.28 mmol), phenylalanine ethyl ester hydrochloride (6.77 g, 37.28 mmol) and triethylamine (37.73 g, 372.82 mmol) in 450 ml THF. The suspension was then refluxed for 72 h to provide complete substitution of the P-Cl bonds. After filtration, the filtrate was concentrated under reduced pressure to yield a yellowish oil. This oil was redissolved in 7 mL THF and then transferred to a dialysis tube, to perform a dialysis (50kDa cut off) versus water for 8 h, ethanol for 12 h and finally THF to redissolve the precipitated solid. The solvent was removed in vacuum to yield 9.17 g (45 %) of a yellowish solid. T_g= 34 - 39 °C Melting point= 205 °C (heat rate 10 K/min) **^{1}H NMR** (CD_2Cl_2, 500.17 MHz): δ = 0.37 - 1.24 (br, 12 *n* H, $[\mathrm{NP(PheOCH_2C}\mathit{H}_3)]_n$, $[\mathrm{NP(NHCHCH(C}\mathit{H}_3)_2)]_n$, $[\mathrm{NP(ValOCH_2C}\mathit{H}_3)]_n$), 1.45 - 1.77 (br, 1.3 *n* H, $[\mathrm{NP(N}\mathit{H})_2]_n$), 1.92 - 2.41 (br, 1 *n* H, $[\mathrm{NP(NHCHC}\mathit{H}\mathrm{(CH_3)_2)}]_n$), 2.72 - 3.60 (br, 3 *n* H, $[\mathrm{NP(NHCHC}\mathit{H}_2\mathrm{C_6}\mathit{H}_5)]_n$, $[\mathrm{NP(NHC}\mathit{H}\mathrm{CH(CH_3)_2)}]_n$), 3.49-5.25 (br, 4.3 *n* H, $[\mathrm{NP(NHC}\mathit{H}\mathrm{CH_2C_6H_5)}]_n$, $[\mathrm{NP(ValOC}\mathit{H}_2\mathrm{CH_3)}]_n$, $[\mathrm{NP(PheOC}\mathit{H}_2\mathrm{CH_3)}]_n$), 6.35 - 8.13 (br, 5 *n* H, $[\mathrm{NP(NHCHCH_2C_6}\mathit{H}_5)]_n$). **^{31}P{^{1}H} NMR** (CD_2Cl_2, 121.5 MHz): δ = −1.3 (br, *n* P, $[\mathrm{N}\mathit{P}\mathrm{(PheOEt)(ValOEt)}]_n$).

2.2 Formation of Polymer Films

To 2.55 g of the polymer, the corresponding amount of TEC (318.8 mg for 12.5 w% and 382.5 mg for 15 w%) was added. Afterwards everything was dissolved in 12.75 mL CH_3Cl. The clear yellow solution was then transferred into a Petri dish with a diameter of 10.8 cm and the solvent was allowed to evaporate over a period of three days. The clear flexible film was then removed from the Petri dish and placed in a vacuum oven at 80 mbar at 25 °C for further three days.

2.3 Degradation Study

The final samples for the degradation study were formed by using a 6 mm hole punch followed by the determination of macroscopic morphology, diameter and mass change. The degradation rate of the polymer films was tested at 50 °C in water and two different HEPES buffer solutions (pH value 6.8 and 7.4). The sample removal time was once a week. In the same step the media of the other samples were exchanged. In addition to the analysis mentioned before, the pH value of the water was tested after the first week and of the two buffer solutions after each medium change.

3 Results and Discussion

3.1 Material Synthesis

The aim was to find a flexible and degrading material with a glass transition temperature in the range of the body temperature, which should enable a specific drug release. Different ratios of amino acids, chain lengths and amounts of TEC were tested in screening tests, resulting in phenylalanine- / valine ethyl ester co- substituted polyorganophosphazene ($PPZ^{PheOEt/ValOEt}$) with 12.5 or 15.0 w% TEC presented in this study. These compositions showed the best properties concerning flexibility and glass transition temperature (T_g = 34 - 39 °C). Higher amounts of phenylalanine ethyl ester led to a more brittle polymer, whereas higher amounts of valine ethyl ester and TEC led to a more wax like polymer.

3.2 Degradation Study

3.2.1 Influence in pH Value

As the pH value has a big influence on the degradation behaviour of polyphosphazenes [9], the pH values of the media after several sample removals were measured. Figure 1 shows a constant pH value in case of every buffered media over the entire degradation experiment, which indicates that the buffer capacity over the medium change period is sufficient to intercept possible degradation products. On the other hand, the pH values of the water media show that acidic decomposition products are formed, which in turn significantly lower the pH value from roughly 5.4 to 3.8. Since all side groups and the TEC are esterified, and the degradation products of the backbone are expected to yield ammonium phosphate, we assume that traces of ammonium salts, e.g., Et_3NHCl, from the synthesis process may contribute to the increase of the pH value. This assumption is supported by NMR spectra of the final polymer which always display traces of Et_3NHCl. As soon as protons are available, the ester bonds can be hydrolysed, which leads to carboxylic acids that also effect the pH value.

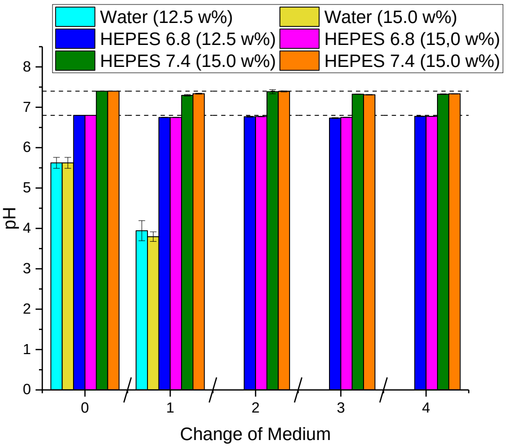

Figure 1: pH value of water, HEPES 6.8 and 7.4 after each medium change, “0” indicates media without sample.

3.2.2 Morphological Characterization

3.2.2.1 Macroscopic Structure

The samples became colourless in water within a week but kept their morphology. After two weeks their size decreased in solution as well as after drying, which indicates a loss in their matrix structure. Between the second and third week of the degradation in water, the samples began to swell dramatically. This peaked after four weeks, where the samples were not visible anymore. Samples behaved differently in the buffer solutions. While immersed, they also lost the transparency, however, it was restored after drying. Since dried samples were sticking to the surface of the glass vials, data points for assessing mass were only available for a few degradation points. However, Figure 4 clearly shows that, in contrast to the samples in water (Figure 2 and 3), there was only minor swelling after three weeks. Samples still remained circular in shape after four weeks in the buffer media, also indicating a slower degradation compared to water.

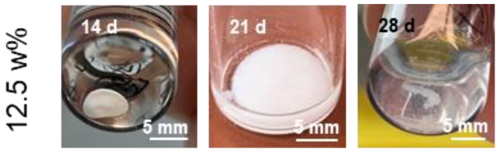

Figure 2: 12.5 w% TEC samples in water after specified incubation times.

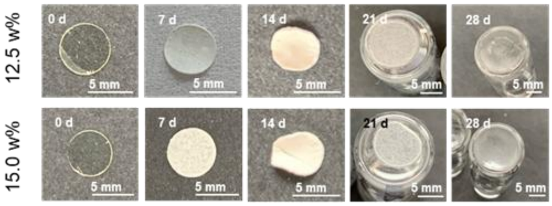

Figure 3: Dried samples after degradation in water at specified incubation times. 12.5 w% TEC content samples in top row, 15 w% TEC samples in bottom row.

Figure 4: Dried HEPES samples after specified incubation times. Left and mid sample: 12.5 w% TEC; Degradation in HEPES 6.8. Right sample: 15w% TEC; Degradation in HEPES 6.8.

3.2.2.2 Diameter and Mass Loss

In accordance with the photos, there was a loss in mass and diameter observed (Figure 5 and 6). There is no significant difference in the diameter loss in dependence of the TEC content within the margin of error. The samples with a lower amount of TEC showed an even higher loss in the diameter. This might be due to the fact, that the determination of the diameter is difficult, which also results in a quite high standard deviation for this value. Samples with a higher amount of TEC showed a higher mass loss. Taking into account, that TEC may be released rather quickly from the samples, these findings came as expected results.

The NMR spectra of the water after degradation of the samples show signals of TEC, which supports this assumption. The swelling of the samples continued to increase during the study. This indicates a further decrease in weight after the second week, however, it was not possible to measure it, since the removal from the vial was not possible without breaking the samples.

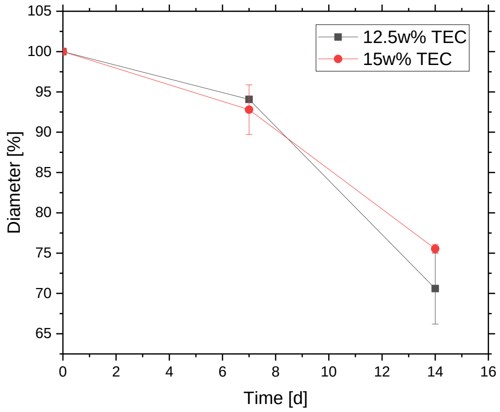

Figure 5: Diameter loss of dried samples, after degradation in water.

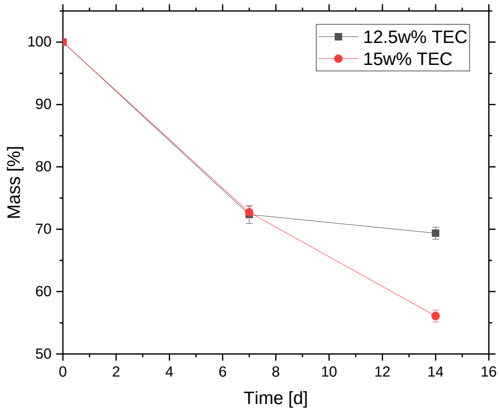

Figure 6: Mass loss of dried samples, after degradation in water.

4 Conclusion and Outlook

We have screened various compositions for the synthesis of an amino acid polyorganophosphazene with TEC as plasticizer, to form a flexible, degradable material. Furthermore, we showed the possibility to form polymer films of this material and investigated the degradation in three different media. The results clearly indicate, that the pH value has a high influence on the degradation rate. Samples in water, which has a lower pH value than the HEPES buffer solutions, degraded much faster and gave indication of nearly complete degradation within four weeks at 50 °C. On the other hand, the capacities of both HEPES buffer solutions were sufficient to buffer the acidic decomposition products or impurities. Qualitative analysis of the samples that degraded in buffer solution also showed no significant degradation within the 4 weeks. Therefore, the degradation behaviour of the polymer presented here significantly depends on the medium. Whether a difference in pH value between 6.8 and 7.4 also shows a difference in degradation behaviour, will be subject to further investigations.

Author Statement

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