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Enzymatically catalyzed degradation of biodegradable polymers investigated by means of a semiconductor-based field-effect sensor

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Abstract

A semiconductor field-effect device has been used for an enzymatically catalyzed degradation of biopolymers for the first time. This novel technique is capable to monitor the degradation process of multiple samples in situ and in real-time. As model system, the degradation of the biopolymer poly(D,L-lactic acid) has been monitored in the degradation medium containing the enzyme lipase from *Rhizomucor miehei*. The obtained results demonstrate the potential of capacitive field-effect sensors for degradation studies of biodegradable polymers.

Keywords: Field-effect sensor, enzymatic (bio)degradation, poly(d,l-lactic acid, in-situ monitoring, impedance spectrscopy

1. Introduction

An accurate prediction of degradation kinetics of biodegradable polymers is essential for the application of such materials in biomedical products. The degradation rate is defined not only by the chemical nature of the degradable backbone and physico-chemical properties of the polymer [1–3], but also the physico-chemical parameters of the degradation environment [4] or the addition of compounds for drug-release applications can have an impact on the

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degradation kinetics [5]. As a consequence, extensive studies on the mechanism and influencing factors are inevitable to understand the degradation process in detail. Common techniques for the study of a degradation of biopolymers, such as gel permeation chromatography, weighing of the mass loss, or nuclear magnetic resonance [6,7], are costly and laborious and consequently, make a total investigation an unfeasible endeavor due to the limited throughput of test samples. Recently, a sensor-based technique utilizing semiconductor field-effect devices has been introduced for degradation monitoring of biopolymers [8]. These devices consist of an electrolyte-insulator-semiconductor (EIS) structure and are able to detect not only interfacial potential changes induced by ions, enzymatic reactions or charged macromolecules [9–12], but also changes in thickness or permittivity of polymer layers deposited on EIS surfaces. Since this approach provides an electrical signal indicating the state of the degradation process, it is capable to monitor the degradation process of a large number of samples in-situ and in real-time.

Poly(lactic acid) (PLA) is one of the most important biodegradable polymers for biomedical applications. One major reason is the fact that PLA degrades to lactic acid, which is a natural component of the anaerobic metabolism of animal and human bodies. Thus, it can leave the organism via a natural pathway. Due to the exceptional importance of PLA, it was chosen as a model polymer for the present work. The functional group of PLA is an ester, which is degraded by means of a hydrolytic splitting in the presence of water. It is known that the rate of this splitting process can be altered by a large number of factors such as the surrounding pH, temperature, ionic strength or enzymes present in a biological environment. In the present work, the enzymatically-catalyzed degradation of PLA was monitored by the field-effect sensor. The degradation process was accelerated and triggered by means of the enzyme lipase from *Rhizomucor miehei* (LipaseRM) [13].

2. Materials and methods

2.1. Sensor structure and signal read-out

The developed sensor consists of a p-doped silicon substrate with an insulating layer of 30 nm SiO_2 and 60 nm Ta_2O_5 on top, and 300 nm Al on the rear-side forming an ohmic contact. The polymer under investigation was applied on the insulating layer as a thin film by means of a spin-coating method (see Fig. 1). The thickness can be adjusted by varying the concentration of the polymer in the solvent as described in [8]. The sensor surface covered
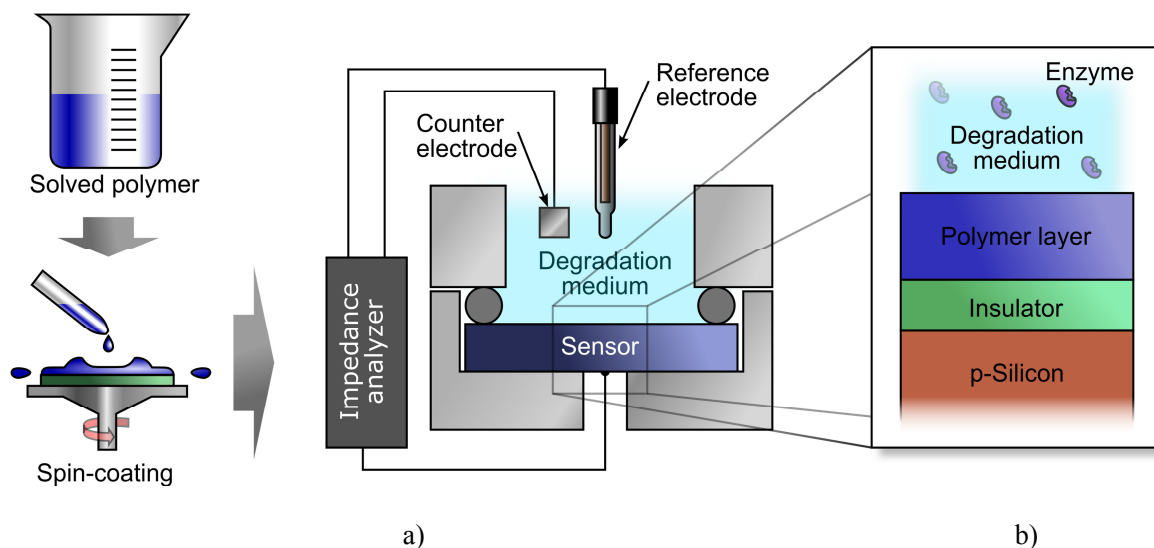

Fig. 1: Illustration of the experimental setup using a semiconductor field-effect sensor for monitoring polymer biodegradation (a), and detailed schematic of the sensor structure covered with a polymer layer (b).

with the polymer film was sealed with an O-ring in a home-made measurement cell containing the degradation medium. The sensor signal was read-out using an impedance analyzer (IM6, Zahner Elektrik GmbH, Germany). A DC bias potential of -2 V vs. the Ag/AgCl reference electrode was applied to the sensor structure to operate the device in the accumulation region. In this working mode, the capacitance of the field-effect sensor is independent on the applied voltage and defined by the gate capacitance. Further details on the relation of the working mode and the bias potential as well as the functional principle of the field-effect device in general is described elsewhere [14]. For the read-out, an AC signal with frequencies of 0.1 Hz to 1 MHz and an amplitude of 20 mV is superimposed in order to determine the complex impedance of the system, including sensor, polymer layer and electrolyte. A full impedance spectrum was captured every hour.

2.2. Polymer and enzyme

The experiments on the enzymatic degradation of the polymer were carried out using the commercial poly(D,L-lactic acid) Resomer® R202H (Evonik Röhm GmbH, Germany), with an isotactic order of the two enantiomers L- and D-lactic acid and a mass-average molar mass of Mw=10,000–18,000. The polymer was solved in methyl ethyl ketone (MEK) with a concentration resulting in a layer thickness of 500 nm after spin-coating. The covered sensors were mounted in separate measurement chambers and were exposed to different degradation media composed of either the pure pH 7 buffer (Titrisol®, Merck KGaA, Germany) or the buffer containing the enzyme LipaseRM (Palatase® 20000L, Novozymes A/S, Denmark). The enzyme was diluted 1:500 in pH-buffer solution and both degradation media were adjusted with respect to the conductivity by adding KCl.

3. Results and discussion

Figure 2 shows the normalized time-resolved sensor signal derived from the captured impedance spectra at a fixed frequency of 100 Hz. The signal was normalized to avoid sensor-related variances of the measured impedance caused by tolerances of the insulator thickness and sensitive area.

The impedance signal of the sensor exposed to pH 7 buffer exhibits an initial phase during which the impedance decreases until it reaches an almost steady condition. This behavior is similar to former studies on polymer degradation in pH 7 using this sensor technique. This process can be due to water penetration or related ion diffusion into the polymer bulk. Strictly speaking, it is not associated to degradation in chemical terms but gives information about fundamental processes that mainly define the degradation kinetics. In this respect, the polymer exposed to pH 7 exhibits slow degradation indicated by the high level of the sensor signal even after 12 days of exposure. In
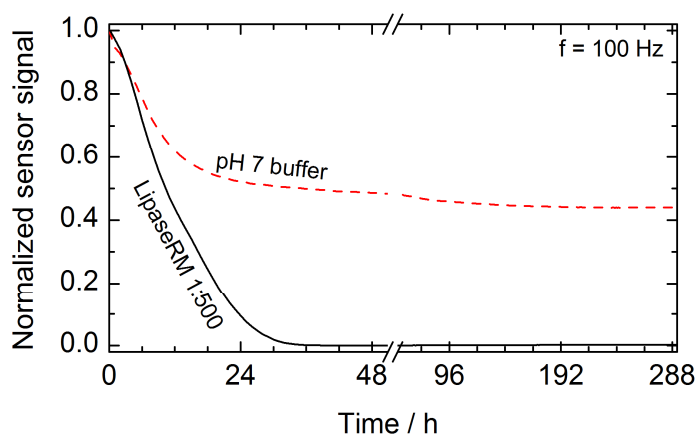

Fig. 2: Normalized signals of sensors exposed to media composed of either pure pH 7 buffer or buffer containing the enzyme LipaseRM.

contrast, the signal of the polymer exposed to the medium containing the enzyme showed a monotonic decrease of the sensor signal and reached the level of the bare sensor after 36 hours.

4. Conclusion

A detailed knowledge concerning the degradation behavior of biodegradable polymers is essential for an expedient and save usage. In this respect, external influences need to be identified and investigated in detail. The successfully performed experiments on the enzymatically catalyzed degradation of poly(D,L-lactic acid) demonstrate the potential of capacitive field-effect sensors as a novel and promising tool for the real-time, in-situ electrical monitoring of degradation kinetics of polymer layers. Its capability to monitor a large number of samples in-situ at the same time facilitates performing extensive studies on multiple parameters without great effort.

Acknowledgements

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