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Degradation Mechanisms of Biodegradable Poly(*DL*-lactide-*co*-glycolide)1000 Diacrylate Network as Studied by Proton Solid-State Flow NMR Relaxometry

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Summary: Degradation mechanisms of biodegradable photo-polymerised poly-(*DL*-lactide-*co*-glycolide)1000 diacrylate {[(*DL*-LA/GA)DA]} matrices or films were studied by proton solid-state NMR relaxometry. A flow NMR unit cell was developed for this purpose. The (*DL*-LA/GA)DA-network was degraded in PBS buffer solution of pH = 7.4. A real-time proton NMR method provided information about the staged break down of the network during continuous circulation of the buffer solution through the NMR tube. The current study shows that degradation of the network proceeds in three stages: 1 - extraction of a sol fraction that causes substantial immobilization of the material, 2 - scissions of network chains producing network defects without formation of extractable products, and 3 – finally, chain scissions that cause formation of a sol fraction and complete degradation of the material. It was shown that the [(*DL*-LA/GA)DA-network was composed of rigid and viscoelastic domains on all stages of degradation. This heterogeneity could be due to heterogeneous spatial distribution of network junctions in the initial network and/or nano-scale phase separation of polyacrylate chains that form multifunctional network junctions and poly(lactide-*co*-glicolide) network chains. A combination of bulk and surface erosion both on nano- and macroscopic scales could explain the observed degradation mechanisms. It is shown that knowledge of weight loss upon hydrolytic degradation is not sufficient for understanding degradation mechanisms in the relation to functional properties of this type of hydrogels.

Keywords: degradation; hydrogels; molecular mobility; network heterogeneity

Introduction

Biodegradable crosslinked polymers are increasingly being exploited as controlled drug delivery matrices.[1] This interest is driven in part by the prospect that biodegradable polymers or materials would obviate the need for surgical intervention to remove the drug eluting implant after the drug delivery period. There are several ways in which biodegradation can be studied.[2] Frequently used methods to monitor degradation are gravimetric weight loss and pH increase. Less information is available about break down of the photo polymerised networks on a nanoscopic and molecular levels. Solid state NMR is extensively used to examine the structure of polymer networks both during and after the photo polymerisation process.[3–5] The aim of this study is to investigate how NMR may be used to analyse degradation of model biodegradable acrylate-based networks on a molecular level with the ultimate intention to understand how network degradation affects drug diffusivity and release, which will be topic of our further studies.

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Experimental Part

A 50 wt % solution of poly(*DL*-lactide-*co*-glycolide)1000 diacrylate [(*DL*-LA/GA)DA] in tetrahydrofuran was applied onto a glass plate using a coating doctor blade designed to give 100 μm thick wet coatings. This wet film was cured under nitrogen atmosphere with a UV dose of 1 J/cm^2 from a D-bulb. The curing was performed at 22 °C on a conveyor belt moving at a speed of 20 m/s. The resulting cured and dried film had a thickness of 50–60 μm. The conversion of acrylate groups, which was determined by FTIR from the intensity of acrylate specific band at 1635 cm^{-1}, was above 90%. The film was degraded at 37 °C in an aqueous phosphate saline buffer solution (PBS) of pH = 7.4. The buffer solution was prepared by dissolving 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl and 1.15 g $NaHPO_4$ in 1 litre of distilled water. For NMR experiments, D_2O (99.9% deuterons) was used for preparing the buffer solution.

The cured film (~400 mg) was placed at the bottom of the NMR tube designed for flow NMR experiments (Figure 1). In order to avoid flushing sample out of the tube, a glass filter with pore size of approximately 100 μm was placed on top of the samples. The filter was tightly fitted in a 9 mm NMR tube using a Teflon tape winded on the edge of the filter. The buffer solution flowed into the tube through an inlet tube of 5 mm diameter. The inlet tube was tightly fitted to the NMR tube of 9 mm diameter using thread on the upper part of the NMR tube and a plastic nut. The lower part of the inlet tube was positioned slightly above the filter surface. The buffer solution flowed out of the NMR tube through the outlet on the upper part of the NMR tube. Inlet and outlet of the NMR tube were connected to an HPLC pump. Approximately 125 ml of the buffer solution was circulated at a speed of 1 ml/min via an HPLC pump (Figure 1). In order to avoid an over-pressure and absorption of atmospheric water vapour by D_2O of the buffer solution, a locker with dry nitrogen was used. The cured film in the NMR tube was positioned inside the detection volume of an NMR spectrometer. Due to large volume of the buffer solution in comparison to the detection volume of the spectrometer (≈0.8 vol %), hardly any sol fraction that was formed upon extraction and degradation was detected.

Proton NMR transverse magnetization decay of the sample, T_2 relaxation decay, was measured as a function of degradation time at 37 °C with a low-field NMR spectro-

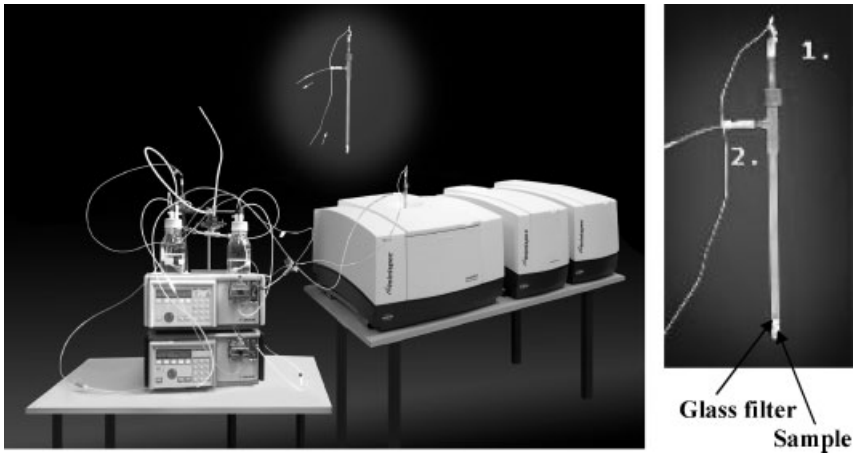

Figure 1.
Picture of experimental set up that was used for the flow NMR relaxation study. Flow-NMR tube is shown on the right side of the Figure. Numbers 1 and 2 indicate inlet and outlet of the tube, respectively.

meter Bruker Minispec MQ-20 operating at a proton resonance frequency of 20 MHz. A combination of the solid-echo and the Carr-Purcell-Meiboom-Gill pulse sequences was used for recording T_2 decays. Since the same receiver gain was used for all experiments, the amplitude of the solid echo can be used as a measure of weight loss (a decrease in the amount of hydrogen atoms in the residual hydrogel) as a function of degradation time.

Results

The Amount of Residual Hydrogel Determined by Weighing and from a Decrease in NMR Signal Amplitude

The weight loss of (*DL*-LA/GA)DA-network is monitored in time (Figure 2). Approximately 10% weight decrease occurred during the first few days after exposure of the film to the buffer solution. This decrease is apparently caused by extraction of a soluble fraction of the film that was present due to not exhausted conversion of acrylate groups. Over the next 20 days hardly any weight loss was observed. After that time, the weight of the film started to decrease continuously and it completely degraded after 35–40 days of its exposure to the buffer solution. Time dependence of weight loss as determined by NMR is similar to that of the extraction study. However, the degradation rate in the NMR tube is five times slower due to approximately 10 folds larger volume of the buffer solution per mass unit of the sample in the NMR experiment. Both experiments do not provide information about changes in molecular structures that occur during degradation, especially during time when no substantial weight loss is observed.

Changes in Molecular Mobility and Network Structure During Degradation as Determined by Proton NMR T_2 Relaxation

T_2 relaxation is highly sensitive to the physical state of materials. Rigid materials/phases have T_2 value of ~10–20 μs, for network chains in rubbery materials ~0.1 ms $\leq T_2 \leq$ 2 ms, for network defects and oligomers T_2 exceeds 10 ms.[4] In the case of large molecular and/or macroscopic heterogeneity of the material, distinct T_2 relaxation components with *widely differing* T_2 decay times have been observed.[3,4] Analysis of changes in molecular mobility can also provide us with information about changes in the network structure caused by prolonged exposure of the network to the buffer solution.[3] Molecular mobility of network chains in swollen gels is largely

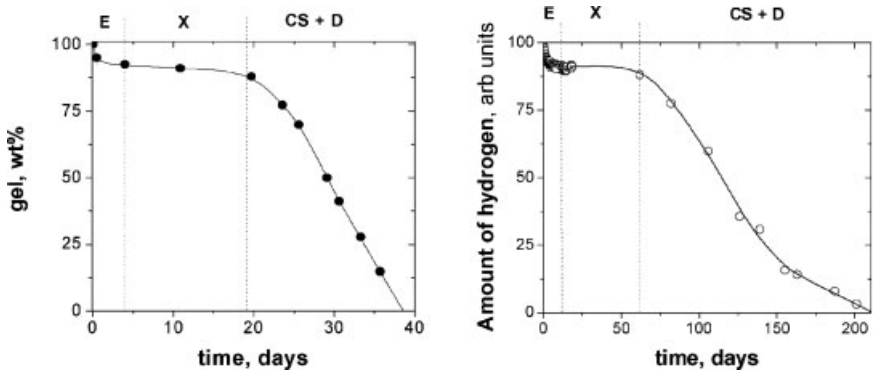

Figure 2.
Weight loss of (*DL*-LA/GA)DA-network in the buffer solution at 37 °C, as determined by extraction - (left), and NMR - (right). Dotted lines indicate different stages of degradation: extraction (**E**); chain scissions followed by extraction of degradation products causing complete degradation of the film (**CS + D**). Molecular changes between stages **(E)** and **(CS + D)** are not clear from the extraction and NMR data on this Figure.

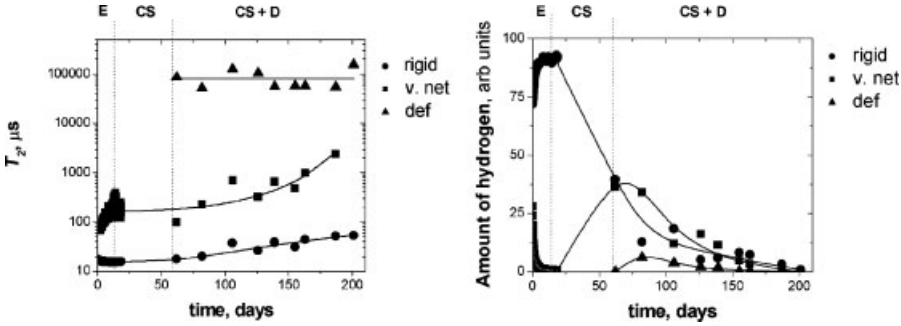

Figure 3.
(Left) - Proton T_2 relaxation time for rigid fraction, viscoelastic network chains and highly mobile network defects in the hydrogel as a function of degradation time. (Right) - The amount of these hydrogel fractions normalized to the amount of initial material as a function of degradation time. Dotted lines indicate three different processes that occur upon degradation time: (E) – extraction of soluble fraction; (CS) – chain scissions causing break down of network chains without formation of soluble products; (CS + D) - chain scissions followed by extraction of soluble degradation products.

decoupled from that of network defects resulting in a major distinction in the T_2 relaxation behaviour of network chains and network defects. The relative intensity of these relaxation components in swollen networks could be used for estimating the fraction of network defects.[3]

Changes in molecular mobility in (*DL*-LA/GA)DA-network, as determined by T_2 relaxation time, are shown as a function of degradation time in Figure 3. The T_2 relaxation during first two stages of degradation (E + CS) can be described with two components whose characteristic decay times (T_2^{rigid} and $T_2^{\text{v. net}}$) differs by one order in the magnitude. This means that two types of polymer chains and/or chain fragments are present in the sample. Values of T_2^{rigid} and $T_2^{\text{v. net}}$ are typical for rigid domains in multi-phase polymers and viscoelastic network chains, respectively.[3,6] A third relaxation component with very long decay time (T_2^{def}) appears during the last stage of degradation (CS + D). Value of T_2^{def} is typical for network defects that are loosely attached to the network.[4] The relative fraction of these relaxation components, % T_2^{rigid}, % $T_2^{\text{v. net}}$ and % T_2^{def}, represents the weight fraction of the rigid fraction, the viscoelastic network chains and the highly degraded network fragments in the residual hydrogel (Figure 4).

Discussion

The network structure of the hydrogel is highly heterogeneous from the beginning to the end of the degradation, as it is concluded from largely heterogeneous molecular mobility in the sample. The presence of a rigid fraction until the end of degradation of the (*DL*-LA/GA)DA-network suggests large spatial heterogeneity (domain-like morphology) of the initial material. Large network heterogeneity was also previously observed at intermediate stages of

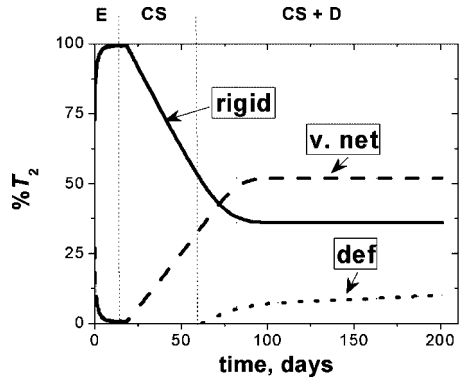

Figure 4.
The relative amount of rigid fraction, viscoelastic network chains and highly mobile network defects in the residual hydrogel as a function of degradation time.

UV-curing of diacrylates oligomers.[5] The other reason for largely heterogeneous structure of the hydrogel during all stages of the degradation could be the following. Upon extraction, lactide-*co*-glycolide network chains and acrylate multi-functional network junctions would tend to be separated on the molecular level because of their intrinsic immiscibility. This might cause additional network immobilization. It can be suggested that the molecular scale heterogeneity of the initial (*DL*-LA/GA)DA-network largely determines its degradation mechanism.

Different Stages of Degradation Process

Results of NMR and extraction studies suggest that the degradation process can be divided in *three stages* with different changes on molecular level occurring upon degradation. These stages are shown as schematic drawings in Figure 5.

Stage 1: (0 ⇒ 6 days) - **E**xtraction. A few hours after flow of the buffer solution into the NMR tube, the swollen hydrogel contains 72% rigid and 28% of a semi-rigid fraction (T_2^{rigid} and $T_2^{\text{v.net}}$ relaxations, respectively) (Figure 3). During the first stage, the weight of the film decreases by approximately 9–10% due to extraction of a sol fraction (Figure 2). At the same time, the amount of mobile fraction decreases from 28% to 0.5% (Figure 3). Thus, approximately 15%–20% of the network is largely immobilized upon extraction of the sol, which plasticisizes some network fragments. Moreover, nano-scale reorganizations during extraction might cause an increase in the strength of physical interactions between poly(lactide-*co*-glicolide) network chains and better nano-scale separation of polyacrylate chains from poly(lactide-*co*-glicolide) network chains. This also could cause additional immobilization of the material. **Stage 2:** (6 ⇒ 60 days) - **C**hain **S**cissions. During this stage, the amount of semi-rigid (viscoelastic) fraction increases to 50% at the expense of the rigid fraction, whereas no significant weight loss of material is observed. Thus, scissions of network chains cause formation of network defects during the second stage. **Stage 3:** (60 ⇒ 200 days) - **C**hain **S**cissions and extraction of **D**egradation products. Molecular mobility in the rigid fraction gradually increases, which is caused by degradation processes affecting this fraction. In addition to the rigid fraction and viscoelastic network chains, small amount of highly mobile chain fragments appears

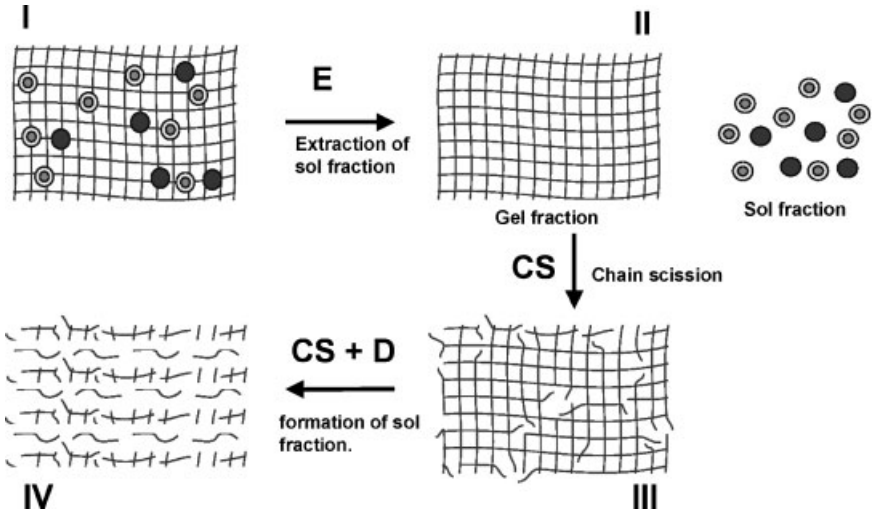

Figure 5.
Schematic drawing of the three stages of degradation of the (*DL*-LA/GA)DA-network.

60 days after exposure of the hydrogel to the buffer solution. The relative amount of the highly mobile (soft) fraction is nearly constant during the last stage of the degradation, i.e. approximately 10% of the residual hydrogel. This fraction originates from loosely attached highly degraded fragments. ''*Three phases*''-like molecular mobility is observed until the complete degradation of the network. The ratio *rigid: viscoelastic chains* of approximately 50 : 50 w/w% remains nearly constant during the final stage of the degradation. Since the rigid fraction remains until the end of the degradation process, this suggests that this fraction largely influences the degradation rate, as determined by weight loss, due to strongly hindered diffusion of water molecules into rigid domains and degradation products out of these domains. It might be also suggested that rigid domains degrade from the surface layer toward the inner part (surface erosion of rigid domains). In addition to the nano-scale heterogeneity of the degradation process, differences in the degradation rate of the skin layer and core part of 50–60 μm thick film can also contribute to the complex mechanism of the degradation. Thus, a combination of bulk and surface types of erosion both on nano- and macroscopic scale could explain the observed degradation mechanisms.

Conclusions

The real-time flow NMR study provided information about the complex mechanisms of hydrolytic degradation of the (*DL*-LA/GA)DA-network. A notable conclusion is that there is evidence of large network break down prior to weight loss, which should have an impact on mechanical properties, drug release behaviour and other properties. This study is the first step in determining mechanisms of drug release in a biodegradable network. It is suggested that network heterogeneity can be largely explored and exploited for controlling the rate of drug release and for staged release of a few drugs.

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