Influence of the Degradation Mechanism of Hydrogels on Their Elastic and Swelling Properties during Degradation

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ABSTRACT: The aim of this study was to investigate the degradation of hydrogels that degrade through different mechanisms. As models, two types of dextran-based hydrogels were investigated. Dextran methacrylate (dex-MA) hydrogels that are degraded by entrapped dextranase were used as a model for hydrogels that degrade by hydrolysis of the polymer backbone. Dextran hydroxyethyl methacrylate (dex-HEMA) hydrogels were used as a model for hydrogels that degrade by hydrolysis of the cross-links. The degradation was characterized rheologically and analytically by determination of the degradation products and by the swelling behavior of the hydrogels. The degradation kinetics as observed from the various characterization techniques were distinctively different and depended on both the composition of the hydrogels and the degradation mechanism. Combining all the results, a qualitative understanding could be proposed for the degradation of the polymer networks of both types of hydrogels. The insight obtained can help the development of degrading hydrogels for specific applications and the choice of a suited technique for evaluation of the degradation.

Introduction

Hydrogels are very suited for biomedical application because of their tissue compatibility, mainly caused by their high water content and their soft, rubbery consistency giving them a certain resemblance to living tissue.[1] While nondegradable hydrogels are used in a number of medical applications (e.g., contact lenses, wound dressings) the clinical use of degradable hydrogels has only been recently explored. The most straightforward applications include membranes for the prevention of surgical adhesions and implantable drug delivery systems. The biodegradable drug delivery systems most commonly used are based on dry mixtures of polylactide and polyglycolide formulations. The drawbacks of these systems are the need for organic solvents during preparation and the acidification during degradation.[2] The research on degradable hydrogels tries to overcome these problems.

The degradability of hydrogels is mostly based on hydrolysis of either the cross-links or the polymer backbone. The latter can be achieved by introducing degradable units into the polymer or by bringing the polymer network in contact with a suitable enzyme. Whatever the application, it is extremely important to be able to follow and quantify the degradation in order to evaluate the performance of a hydrogel-based system. This is normally done by quantification of the degradation products, measuring the degree of swelling or the mechanical properties of the hydrogel. [1,3−6] The results of these determinations should be linkable since they all result from the same process and are therefore complementary. An important question however is whether the measured properties really reflect the degradation mechanism and which technique is the most sensitive for this purpose.

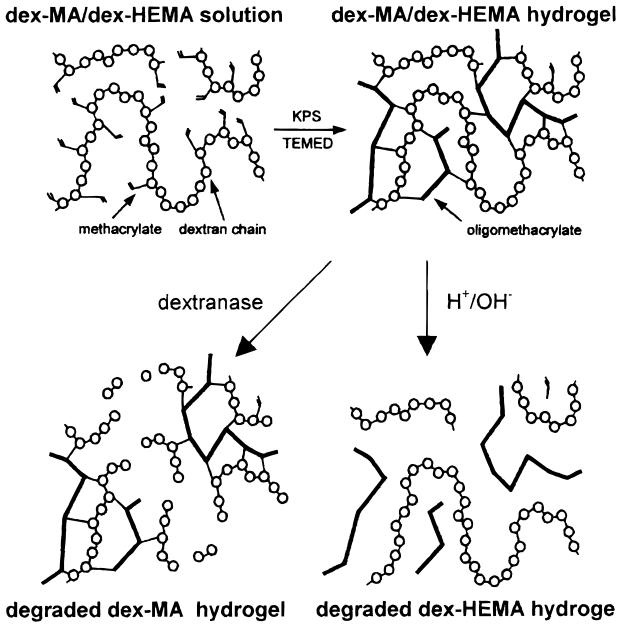

Figure 1. Schematic representation of the formation of the polymer network in dex-MA and dex-HEMA hydrogels. The enzymatic and chemical hydrolysis is also illustrated.

In this study, as models for hydrogels with a different degradation mechanism, two types of dextran-based hydrogels were investigated. As shown in Figure 1, the first model consists of dextran methacrylate (dex-MA) hydrogels that are degraded by dextranase. The dextranase in this study was entrapped within the hydrogel resulting in a bulk degradation. These dex-MA/dextranase hydrogels were used as a model for hydrogels that degrade by hydrolysis of the polymer backbone. The second model consists of dextran hydroxyethyl meth-

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acrylate (dex-HEMA) hydrogels. The hydrolyzable carbonate ester introduced between the methacrylate group and the dextran results in hydrolyzable cross-links.[7,8] These hydrogels dex-HEMA hydrogels were used as a model for hydrogels that degrade by hydrolysis of the cross-links.

The aim of this study was twofold. First was to find out how the degradation mechanism of the hydrogels influenced their mechanical and swelling properties. Second, it was investigated whether the rheological data could be linked with the analytical data on the amount of degradation products released from the hydrogels, by comparison of the observed kinetics.

Experimental Section

dex-MA and dex-HEMA Preparation and Characterization. The dex-MA and dex-HEMA batches were prepared and characterized as described in detail elsewhere.[9−11] The degree of methacrylate (ma) substitution (DS, i.e., the number of ma molecules per 100 glucopyranose units) was determined by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) in D_2O with a Gemini 300 spectrometer (Varian). The DS of the batches used in this study varied from 1.2 to 9.

Preparation of the Dextran-Based Hydrogels. The preparation of both types of hydrogels is based on the same general procedure using a radical polymerization of aqueous solutions of the polymers. These solutions were prepared by dissolving the polymers in buffer (phosphate buffer: 10 mM Na_2HPO_4, 0.02% sodium azide, adjusted with 1 N hydrochloric acid to pH 7.0 or 8.5 for hydrogels with dextranase). The polymerization reagents were *N*,*N*,*N*′,*N*′-tetramethyleneethylenediamine (TEMED; 20% v/v in deoxygenated phosphate buffer, pH adjusted to 8.5 with hydrochloric acid) and potassium persulfate (KPS; 50 mg/mL in deoxygenated phosphate buffer). Adding 50 μL of TEMED solution (per gram of hydrogel), followed after stirring by 80 μL of KPS solution (per gram of hydrogel), started the gelation. All the containers in which gelation occurred had been coated with a poly(ethylene glycol) solution (PEG 20 000 g/mol; 10% in phosphate buffer) to simplify the removal of the hydrogel after gelation. On average, a complete gelation took 90 min at room temperature. Throughout this work the concentration (% (w/w)) of the hydrogels refers to the concentration at cross-linking. Hydrogel slabs for rheology and swelling experiments were prepared by transferring 9 mL of the gelating mixture into 15 mL polypropylene syringes from which the heads were cut. After gelation a part of the hydrogel was pushed out of the syringe and sliced off with a thin wire. When not used immediately, the hydrogel slabs were stored individually at 4 °C in small bags made from Parafilm.

For the preparation of dex-MA/dextranase hydrogels, the enzyme solution (D-1508 Sigma; diluted to 10 U/mL in 5 mM citrate buffer pH 6; one unit will deliver 1 μmol of isomaltose per minute at pH 6 at 37 °C) was added to the dex-MA solution (cooled to 4 °C) prior to the addition of the gelation reagents, as described above. Since only minute amounts of enzyme solution had to be added, no pH shift was observed in the dex-MA solution. The gelating solution (5 mL) was transferred into the syringes and kept at 4 °C for 90 min. Hydrogel slabs were cut and stored overnight in phosphate buffer pH 8.5 to allow swelling under conditions of low dextranase activity (i.e., high pH). Finally, they were transferred to phosphate buffer pH 7 and stored at 37 °C to allow degradation.

Dex-MA/dextranase hydrogels were also prepared in situ between the plates of the measuring geometry of the rheometer. In this case, a 4 cm Petri dish was fixed to the bottom plate to form a reservoir into which the gelling solution was poured. Immediately after that, a distance of 1800 μm between the plates of the geometry (gap) was set, and the gelation occurred at 4 °C. After gelation the excess of hydrogel was removed from the Petri dish. Degradation was started by raising the temperature to 37 °C and by submerging hydrogel and geometry with buffer (5 mM citrate pH 5.5).

Swelling Experiments. To characterize the swelling behavior of the hydrogels, they were weighed immediately after preparation and on several occasions during their degradation. The swelling ratio was calculated by dividing the weight of the hydrogels at equilibrium swelling by their weight after gelation.

Mechanical Characterization of the Hydrogels. For the mechanical characterization of the hydrogels, rheological measurements were performed on an AR1000-N controlled stress rheometer from TA-Instruments. The rheometer was adapted for the measurement of hydrogel slabs by sticking sandpaper to the geometry. The bottom plate was replaced with a Plexiglas plate with a roughened surface that was fixed on the rheometer with clamps. This allowed removing the hydrogels together with the bottom plate, avoiding manipulation of the degrading hydrogels. All measurements were performed with an acrylic top plate (diameter 2 cm) equipped with a solvent trap to avoid evaporation. Unless described otherwise, all experiments were done in oscillation mode at 1 Hz by applying a constant strain of 0.5%. Further details on the method used were described in detail by Meyvis et al. elsewhere.[12]

Results and Discussion

Degradation of Hydrogels through Hydrolysis of the Polymer Backbone. 1. Analytical Characterization of the Degradation. The degradation of hydrogels through hydrolysis of the polymer backbone was evaluated using dex-MA/dextranase hydrogels as a model system. As shown in Figure 1, the endo dextranase, entrapped within the network during polymerization, hydrolyzes the network chains of the polymer network. After hydrolysis of a network chain, the remaining strands (dangling ends) can be further degraded if long enough to fit within the active site of the dextranase.[13] This type of degradation will result in a variety of degradation products that are released from the hydrogels during degradation. Recently, Fransen et al.[14] analytically defined the degradation kinetics of the dex-MA/dextranase hydrogels on the basis of the quantification (determination of reducing oligosaccharides) and identification (by electrospray mass spectroscopy) of the degradation products. The study suggested that the degradation of dex-MA hydrogels by dextranase follows a two-substrate model. The "primary substrate", with a high affinity for dextranase, was identified as chain segments of 18 or more unsubstituted glucopyranose residues. The "secondary substrate", with a much lower affinity for dextranase, consisted of chain segments with 6−18 unsubstituted glucopyranose residues. Chain segments with less than six unsubstituted segments were not degraded in dex-MA hydrogels due to their severe conformational restrictions. In Figure 2a the amount of *reducing oligosaccharides released from the polymer network* during the degradation of two distinctively different dex-MA/dextranase hydrogels is shown. The DS 4.0 dex-MA/dextranase hydrogel degraded relatively quickly with an almost constant release rate of reducing oligosaccharides. After 2 days the DS 4.0 hydrogels disintegrated. A DS 7.0 dex-MA/dextranase hydrogel, on the other hand, initially showed a rather fast release rate of reducing oligosaccharides slowing down as degradation proceeded. The latter hydrogels did not fall apart within the experimental period nor even after several weeks, indicating that large parts of the polymer network were not degradable. The constant release rate of reducing oligosaccharides from the DS 4.0 dex-MA/dextranase hydrogels showed that the dextranase was operating under V_{max} conditions. The gradual decrease in the release rate form DS

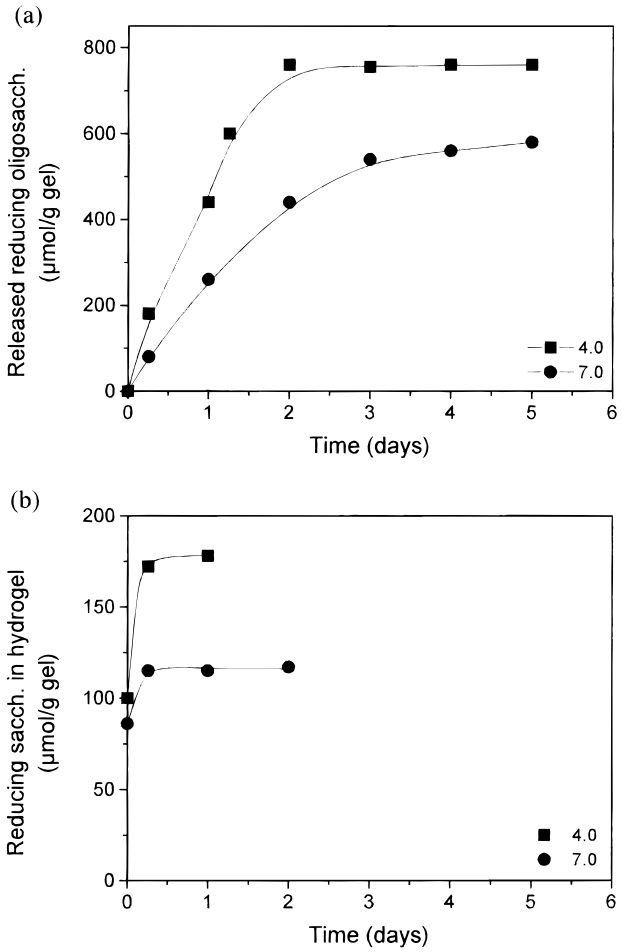

Figure 2. Formation of reducing saccharides in time for dex-MA/dextranase hydrogels (30%; 1 U/g hydrogel) of different DS with **(**a**)** the amount of released reducing oligosaccharides and **(**b**)** the amount of reducing saccharides inside the hydrogels. The data are an average of two independent measurements not differing more than 5% (after Franssen et al. (1999)).

7.0 hydrogels probably indicated a deviation from V_{max} (enzymatic reaction rate at substrate saturation) conditions as degradation proceeded, due to the nondegradable parts of the hydrogels.

Each time dextranase hydrolyzes a network chain a reducing oligosaccharide remains as one of the created dangling ends. Further degradation of these dangling ends will not alter the amount of reducing oligosaccharides bound to the polymer network. Therefore, the amount of *reducing oligosaccharides bound to the polymer network* is related to the number of network chains that were hydrolyzed. Figure 2b represents the evolution of this amount of reducing oligosaccharides as determined by Franssen et al. for the dex-MA/dextranase hydrogels of Figure 2a. A maximum was reached within 10 h from the start of the degradation for both the DS 4.0 and 7.0 dex-MA/dextranase hydrogels. This would mean that after this time the enzyme no longer hydrolyzes network chains but only the dangling ends. These results were clearly inconsistent with the observation that the DS 4.0 hydrogels only fell apart after 2 days.

2. Rheological Characterization of the Degradation. We wondered to what extent the analytical data presented above could be correlated with changes in the mechanical properties of these hydrogels during degradation. Therefore, a rheological investigation of the dex-

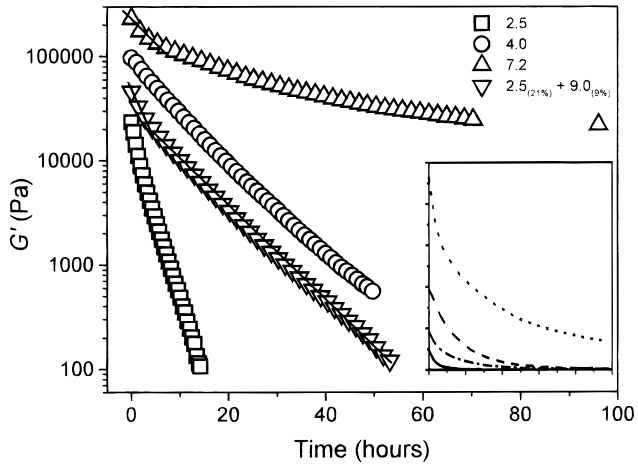

Figure 3. G' as a function of time of dex-MA/dextranase hydrogels (30%; 1 U/g hydrogel) with different DS (2.5, 4.0, 7.2 and a mixture of 2.5 (21%) and 9.0 (9%)) degraded between the geometry of the rheometer in permanent contact with buffer (pH 5.5; 37 °C). For clarity, only a fraction of the points are shown. The data are an average of at least three repeats; the separate degradation profiles differed no more than 5%. The inset shows the degradation profiles during the first 50 h on a linear scale.

MA/dextranase hydrogels using the same conditions (composition, preparation method, temperature, and pH) as Franssen et al. was performed. The G' of the dex-MA/dextranase hydrogels was chosen as a parameter for the evolution of the mechanical properties during degradation. All dex-MA/dextranase hydrogels were cross-linked in situ between the plates of the geometry of the rheometer. For these experiments, a special chamber was constructed, allowing the hydrogel to be in contact with its solvent during degradation. After gelation the degradation was started upon addition of the solvent (citrate buffer pH 5.5) to the chamber and raising the temperature to 37 °C. The rheologically observed degradation profiles are shown in Figure 3. The data were plotted on a semilog plot because a very large decrease in G' was always observed during the initial part of the degradation. Being between the plates of the geometry during the degradation, the hydrogels could not swell freely. This restriction may have slowed down the degradation rate by limiting the mobility of the dextranase and/or the accessibility of substrate within the network.[15] As shown in Figure 3, the DS 4.0 dex-MA/dextranase hydrogels lost the majority of their elasticity after 2 days, which was consistent with the period after which the hydrogel fell apart in the study of Franssen et al. While the amount of reducing oligosaccharides in the network no longer increased after 10 h (Figure 2b), suggesting the end of the hydrolysis of network chains, the rheological data clearly show that for all hydrogels G' dropped considerably over several days. As the G' of the hydrogels could only decrease upon hydrolysis of an elastic network chain, the amount of bound reducing oligosaccharides should have continued to increase over the degradation period. The determination of reducing oligosaccharides bound to the polymer network clearly lacked sensitivity.

A more detailed look at the rheological degradation profiles in Figure 3 reveals that for all dex-MA/dextranase hydrogels studied the mechanical degradation rate (rate of G' decrease) slowed down considerably as degradation proceeded (inset of Figure 3 shows the degradation profiles on a linear scale). This was in

contrast with the continuous release rate of reducing oligosaccharides (Figure 2a), suggesting a constant degradation rate. An explanation can be found in the basics of the rheological measurements. When measuring G' during degradation only the action of dextranase on *elastic* network chains is determined. The release rate of reducing oligosaccharides, on the other hand, reflects the action of the enzyme on all types of dex-MA chains (both elastic and nonelastic) present in the hydrogel. Moreover, upon hydrolysis of an elastic chain, two nonelastic dangling ends are created. These dangling ends can be further hydrolyzed by the enzyme, but this will cause no change in G'. As degradation proceeds more dangling ends were created, and the rheologically observed degradation rate slowed down. Therefore, it was concluded that the rheologically observed degradation is determined not only by the amount of primary and secondary substrate but also by the dangling ends, created upon hydrolysis of an elastic chain. These dangling ends will act as a kind of competitive inhibitor for the hydrolysis of elastic chains by dextranase, slowing down the rheologically observed degradation rate as degradation proceeds. Theoretically spoken, these dangling ends are not true competitive inhibitors, but a cosubstrate whose hydrolysis cannot be quantified using rheology. Some of the degradation profiles in Figure 3 could be fitted with a straight line. Their rheologically observed degradation kinetics therefore appeared to be first order. Knowing from the analytical data that the dextranase has a constant activity during degradation, the apparent first-order kinetics indicated that the competitive inhibition for the degradation of elastic chains was proportional to the amount of elasticity lost throughout the degradation.

The rheological degradation profiles clearly depended on the DS of the dex-MA/dextranase hydrogels. Straight lines could be fitted to the degradation profiles of both DS 2.5 and 4.0. For the DS 7.2 dex-MA/dextranase hydrogels only the first 10 h of the degradation could be fitted with a straight line (indicative line is drawn in Figure 3). The slope of this line was equal to that of the DS 4.0 hydrogels. After these first hours the degradation slowed down more rapidly. The different kinetics observed for low and high DS dex-MA/dextranase hydrogels may be explained using the two-substrate model for the degradation of dex-MA hydrogels by dextranase as developed by Franssen et al. It is very likely that in the DS 2.5 hydrogels all elastic chains consisted of dex-MA with 18 or more unsubstituted glucopyranose residues ("primary substrate"). In the DS 4.0 hydrogels, on the other hand, "primary substrate" as well as elastic chains of dex-MA with 6−18 unsubstituted glucopyranose residues ("secondary substrate") could have been part of the network. This resulted in a slower degradation as can be deducted from the apparent degradation reaction constants (k_{app}) calculated from Figure 3 (1.9×10^{-5} s^{-1} for DS 2.5 and 5.4×10^{-6} s^{-1} for DS 4.0). Further increasing the DS resulted in nonfully degradable hydrogels with elastic chains that, next to nondegradable chain segments (less than six unsubstituted glucopyranose residues), mainly consisted of secondary and some primary substrate. The initial degradation, during which almost 50% of the total elasticity was lost, followed the kinetics of the DS 4.0 hydrogels. It is very likely that after this initial degradation a shortage of substrate occurred because mainly the nondegradable chains remained in the network. As a consequence, no constant k_{app} existed after the initial degradation. Furthermore, the structure of the networks in high DS dex-MA hydrogels may be very dense, probably limiting the mobility of the dextranase within the network.[16] On its turn, this may reduce both the activity of the dextranase and the degradability of the hydrogels.

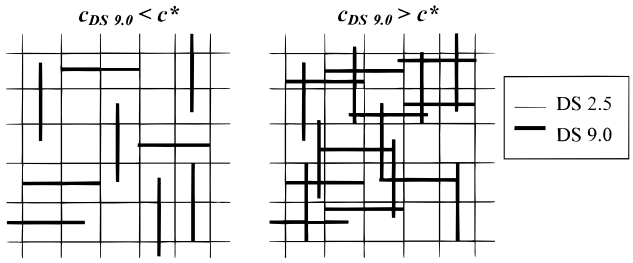

Figure 4. Schematic illustration of the networks of dex-MA hydrogels created by mixing dex-MA DS 2.5 with DS 9.0. The left part shows the network obtained when the concentration of DS 9.0 dex-MA in the hydrogel is beneath c^* and the right part when the concentration of DS 9.0 dex-MA in the hydrogel is above c^*.

Figure 3 also shows the rheological degradation profile of a dex-MA/dextranase hydrogel (30% dex-MA) which was made from two types of dex-MA: DS 2.5 (70% of total dex-MA mass) and DS 9.0 (30% of total dex-MA mass) with a concentration in the hydrogel of respectively 21 and 9%. The concentration (9%) of highly substituted dex-MA was deliberately kept below critical overlap concentration ($c^* = 12\%$ (w/w)) of dex-MA. In this way we aimed to create a network that mainly consists of primary (due to the DS 2.5 dex-MA) and a fraction of secondary substrate (due to the DS 9.0 dex-MA) as schematically illustrated in the left part of Figure 4. The fine lines represent the basis of the polymer network formed by the DS 2.5 dex-MA. The elastic chains between the junctions (whenever lines cross) are primary substrate (one unit of the grid). The thick lines represent the DS 9.0 dex-MA molecules. Having more methacrylate groups per dex-MA molecule, they will introduce some secondary substrate in the network (represented by half a unit of the grid). If the concentration of DS 9.0 dex-MA in the hydrogel had exceeded c^*, these molecules would have been able to interconnect and form a dense network, creating a nondegradable backbone within the hydrogel (illustrated in the right part of Figure 4). The rheological degradation profile of this hydrogel (Figure 3) had an initial k_{app} (1.2×10^{-5} s^{-1}) similar to that of the DS 2.5 dex-MA/dextranase hydrogels. The amount of elasticity lost during these first hours therefore mainly arose from the degradation of parts of the hydrogel having a network structure very similar to that of the DS 2.5 hydrogels. After this initial phase the degradation continues with a k_{app} (5.4×10^{-6} s^{-1}) identical to that of DS 4.0 hydrogels. During this stage the parts of the network consisting of both primary (from the DS 2.5 dex-MA) and secondary substrate (from the DS 9.0 dex-MA) were degraded. The observed degradation kinetics therefore confirmed the existence of a network with a structure similar to that schematically illustrated in the left part of Figure 4.

Generally, it could be concluded that in order to describe the analytically (reducing oligosaccharides) or rheologically observed degradation of dex-MA/dextranase hydrogels different enzymatic models had to be

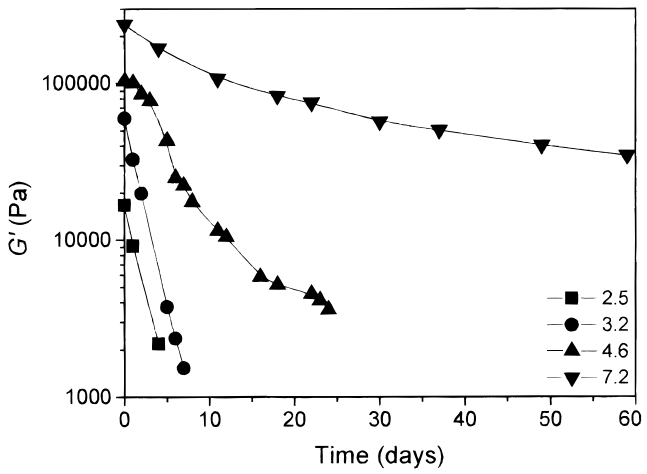

Figure 5. G' as a function of time of dex-MA/dextranase hydrogels (30%; 0.1 U/g hydrogel) of dex-MA with different DS (2.5, 3.2, 4.6 and 7.2); between measurements the hydrogels were stored in buffer (pH 7.0, 37 °C). The data are an average of at least three repeats; the separate degradation profiles differed no more than 7%.

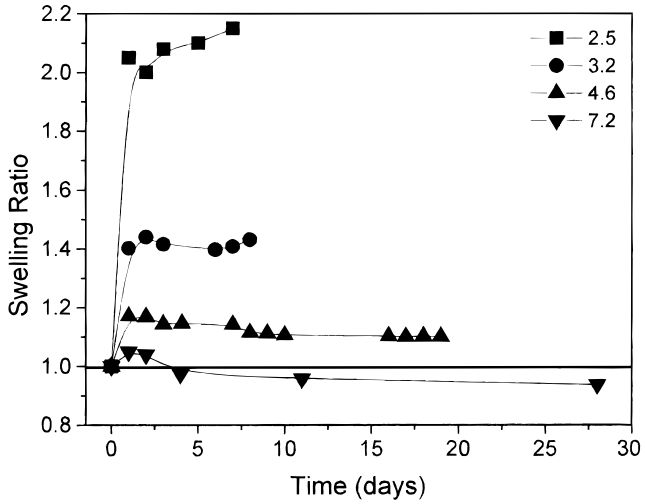

Figure 6. Swelling ratio of dex-MA/dextranase hydrogels (30%, 0.1 U/g hydrogel) with different DS (2.5, 3.2, 4.6 and 7.2) after swelling to equilibrium under conditions of low dextranase activity (point at day 1) and during degradation in buffer (pH 7.0, 37 °C). The data are an average of at least three repeats; the separate swelling profiles differed no more than 5%.

used. While the analytical results could be explained with a one- or two-substrate model, depending on the DS of the dex-MA,[13] the rheological degradation model had to be extended with a competitive inhibitor created upon hydrolysis of a network chain.

All the experiments reported in Figure 3 were performed at a high dextranase concentration and optimal pH for the enzyme and keeping the hydrogels between the plates of the geometry throughout the entire degradation. The latter totally restricted possible swelling of the hydrogels during degradation. Using a much lower dextranase concentration and a higher pH (7.0), slowly degrading dex-MA/dextranase hydrogels were made. Since their degradation could take up to weeks, these had to be removed from the rheometer and stored in buffer between measurements. We wondered whether the same relation between the rheological degradation kinetics and the DS of the dex-MA could be observed on these slowly degrading dex-MA/dextranase hydrogels that were able to swell freely. As can be seen from Figure 5, the rheological degradation profiles revealed similar trends as observed for the fast degrading hydrogels in Figure 3. Hydrogels made from low substituted dex-MA (DS 2.5 and 3.2) showed apparent first-order degradation kinetics with similar degradation rate constants ($k_{app} = 1.1 \times 10^{-6}$ s^{-1} for DS 2.5 and 1.2×10^{-6} s^{-1} for DS 3.2). These values of k_{app} roughly differed by 1 order of magnitude with those calculated for the fast degrading DS 2.5 hydrogels, which was in accordance with the ratio of the dextranase concentrations. From DS 4.6 onward a deviation from first-order kinetics was observed and the degradation slowed down as the DS of the dex-MA increased. Compared to fast degrading dex-MA hydrogels, an extra factor could have caused this deviation. The longer the degradation, the more likely that dextranase was released from the matrix either free or bound to degradation products.[17] This probably explains why the deviation already occurred from DS 4.6 onward. In general, the degradation kinetics observed in Figure 5 confirmed the results of Figure 3 and allowed to conclude that the rheological degradation of dex-MA/dextranase hydrogels was not influenced by keeping the hydrogels in between the plates of the rheometer.

3. Characterization of the Degradation through Swelling. The swelling behavior of hydrogels is another parameter often used to characterize hydrogels. To investigate the evolution of the degree of swelling caused by hydrolysis of the polymer backbone of a hydrogel, several dex-MA/dextranase hydrogels were analyzed during degradation (Figure 6). Since the swelling of a hydrogel is a rather slow process, slowly degrading dex-MA/dextranase hydrogels (same as in Figure 5) were chosen. Under these conditions a better approximation of an "equilibrium swelling" at each stage in the degradation could be obtained. The weight of the hydrogels after cross-linking was taken as the first point (swelling ratio equals unity). The second point was obtained after immersion overnight of the hydrogels in buffer under conditions of minimal dextranase activity (pH 8.5, 4 °C). The swelling ratio after 1 day in Figure 6 therefore represents the equilibrium degree of swelling of the "intact" hydrogels. Knowing this, it was striking to observe that during degradation (from day one onward), independent of the DS, the swelling ratio of the dex-MA/dextranase hydrogels only showed little changes while the G' of the hydrogels decreased considerably. According to the "constrained junction model", the following equation holds:[18,19]

$$G' = (\upsilon - h\mu)RT \qquad (1)$$

where υ is the concentration of elastic network chains, μ the concentration of elastic cross-links, and h a factor ranging between 0 for "affine" and 1 for "phantom" networks. It was shown previously that dex-MA hydrogels, especially with a high DS, probably have cross-links with a high functionality.[12,14] A schematic illustration of the evolution of a network, with cross-links of a high functionality, upon enzymatic hydrolysis of the network chains is given in the left part of Figure 7. As an elastic network chain is degraded, the concentration of elastic network chains (υ) decreases and thereby, according to eq 1, also G'. However, due to the high functionality of the cross-links, the concentration of elastic cross-links will remain the same during a major

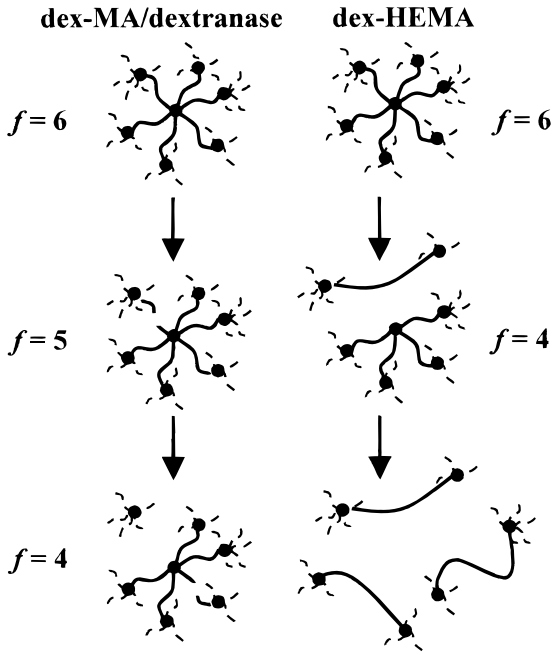

Figure 7. Illustration of the importance of cross-links with a high functionality in the polymer network of dex-MA/dextranase and dex-HEMA hydrogels during the degradation. The functionality of the cross-link after each degradation step is also indicated.

part of the degradation. As a consequence, the average molecular weight between the cross-links is also unaltered, making any extra expansion of the network impossible.

Degradation of Hydrogels through Hydrolysis of the Cross-Links. 1. Analytical Characterization of the Degradation. As a model for hydrogels that degrade through hydrolysis of their cross-links (base-catalyzed hydrolysis of the carbonate ester), dex-HEMA hydrogels were prepared using dex-HEMA with various DS. Franssen et al.[20] analytically characterized the degradation of dex-HEMA hydrogels by determining the amount of dextran released from the hydrogels. As illustrated in Figure 8, a lag time (t_{lag1} and t_{lag2}) in the release, which strongly depended on the DS, was found. The lag time was explained by the fact that all cross-links connecting a single dex-HEMA chain to the network had to be broken before the chain could be released. After the lag time, the release rate was more or less constant and depended slightly on the DS of the dex-HEMA.

2. Rheological Characterization of the Degradation. As for dex-MA/dextranase hydrogels, the evolution of the rheological properties during degradation of dex-HEMA was followed. Since the hydrolysis of the cross-links is a rather slow process, the hydrogels were removed from the rheometer between measurements and stored in buffer. Dex-HEMA hydrogels with a DS of 2.5 and 8.0 and with a dex-HEMA concentration of 30% were used. Figure 9a shows the rheological degradation profiles for the 30% hydrogels together with the observed swelling which will be discussed further on. A much more gradual decrease of G' was observed compared to the rheological degradation profiles of dex-MA/dextranase hydrogels, and therefore linear axes were preferred. Since the hydrolysis of the carbonate ester in dex-HEMA is not limited by any sterical factors, like in the enzymatic degradation of dex-MA (dex-MA

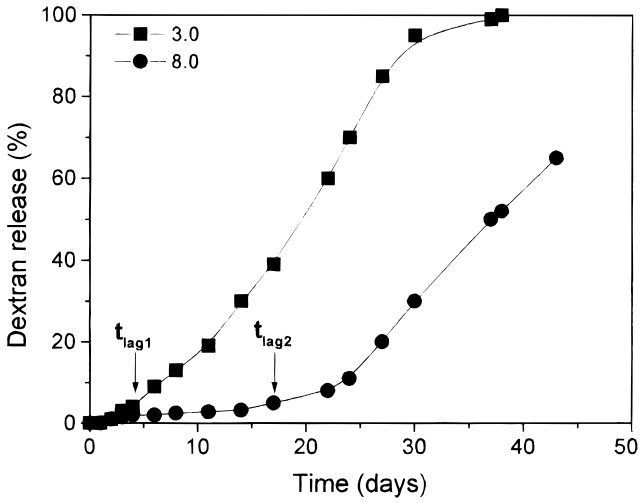

Figure 8. Cumulative release of dextran from dex-HEMA hydrogels (30%) of different DS (3.0 and 8.0) as a function of time. The lag times (t_{lag}) during which no release of dextran was observed are also indicated. The values are a mean of two independent measurements that deviated less than 5% of the total cumulative release (after Franssen et al. (1999)).

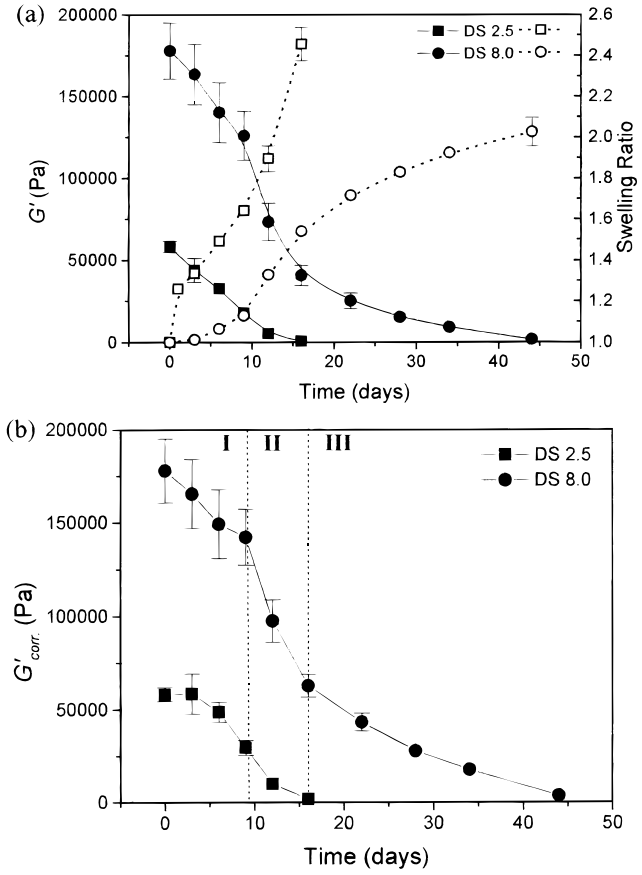

Figure 9. G' and the swelling ratio as a function of time of dex-HEMA hydrogels (30%) of different DS (2.5 and 8.0) **(**a**)** and the G' corrected for the degree of swelling (G'_{corr}) as a function of time **(**b**)**. The profile of the DS 8.0 hydrogels was divided in three sections, each with their own degradation rate. Error bars are not shown when smaller than the symbol.

chains have to fit in the active site of dextranase for enzymatic degradation), even hydrogels with a high DS fully degraded. The shape of the profiles was again DS dependent. In contrast to the dex-MA/dextranase hydrogels, a large amount of swelling took place during

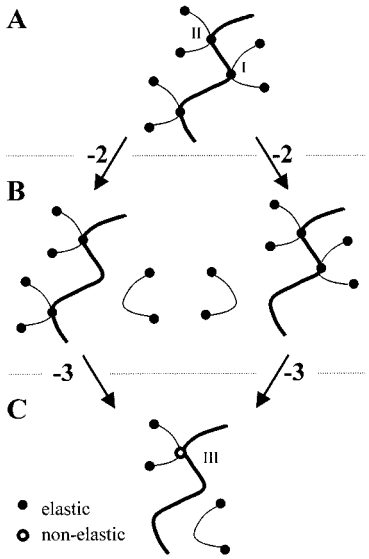

Figure 10. Schematic illustration of the change in elastic network chain concentration upon hydrolysis of a cross-link on a dex-HEMA molecule as a function of the total amount of cross-links present on that dex-HEMA molecule.

degradation. The reason for this difference will be discussed further on.

Introducing the functionality of the cross-links (f), eq 1 can be rewritten

$$G' = v\left(1 - h\,\frac{2}{f}\right)RT \qquad (2)$$

As swelling decreases v, according to eq 2 swelling will decrease G', even when no degradation occurs. Therefore, to evaluate the degradation of dex-HEMA hydrogels through the changes in G', a correction was made for the decrease in G' caused by swelling. A simple proportionality between G' and the polymer concentration of the hydrogels was assumed. This approach was already successfully applied for the rheological characterization of gelatin films.[21] For each G' measured during degradation, a correction factor was defined as the ratio of the initial polymer concentration after cross-linking and the polymer concentration calculated from the associated swelling. Multiplying the G' values by their corresponding correction factors resulted in the profiles represented in Figure 9b. Van Dijk-Wolthuis et al. showed that the hydrolysis of the carbonate ester in dex-HEMA is a pseudo-first-order process.[8] This however was not reflected in the observed rheological degradation profiles. For the DS 8.0 hydrogels, three time regions with a more or less continuous decrease rate of G' could be identified (delimited by the dotted lines in Figure 9b). An initial phase of rapid decrease in G' was followed by a middle phase with an even steeper decrease of G'. In the last phase the decrease rate of G' slowed down. An explanation can probably be found in the net effect that hydrolyzing one cross-link has on the amount of elastic chains in the network. As Figure 10 explains, this net effect depends on the amount of cross-links present on a single dex-HEMA molecule. A simple model for a dex-HEMA molecule within the network, assuming only two methacrylate groups can react to form a cross-link, is shown in Figure 10. The bold line in Figure 10 is our chain of interest. When a cross-link is created between a methacrylate group located in the middle of the chain of interest and one located in the middle of a surrounding dex-HEMA molecule, the created cross-link is tetrafunctional as four elastic chains leave from this cross-link (e.g., cross-link I in Figure 10). The cross-links formed between a methacrylate at the extremities of the chain of interest and a methacrylate located in the middle of another dex-HEMA molecule are trifunctional (e.g., cross-link II in Figure 10) as only three elastic chains (and one dangling end) leave from this cross-link. Consequently, our chain of interest has a trifunctional cross-link at each extremity and one tetrafunctional cross-link in the middle. As illustrated in Figure 10A,B hydrolyzing one cross-link on the chain of interest will cause a loss of two elastic network chains independent of the cross-link chosen. This is also valid when more than three cross-links are present on the chain of interest or if the cross-link has a higher functionality. On the other hand, hydrolyzing a cross-link on the chain of interest when only two cross-links are left (Figure 10B,C) will cause a loss of three elastic network chains. The remaining cross-link (e.g., cross-link III in Figure 10) is no longer elastic but is still part of an elastic network chain. Eliminating this final cross-link will have no effect on the amount of elastic chains in the network. Using this model the observed rheological degradation profiles (Figure 9) can be explained as follows. Since the number-average molecular weight of dex-HEMA equals 19 kDa, one can calculate that the maximum amount of cross-links present on a single dex-HEMA molecule is almost exactly the DS of that chain. Therefore, for DS 8 dex-HEMA hydrogels, the chances are very likely that in the early stages of the degradation more than two cross-links were present on each dex-HEMA molecule. As degradation proceeded, the fraction of dex-HEMA molecules with only two cross-links left increased and the rheologically observable degradation became faster (region II in Figure 9b). Further on in the degradation the fraction of dex-HEMA molecules having only one cross-link left increased, and the rheologically observable degradation slowed down (region III in Figure 9b) because the degradation of these cross-links had no effect on the elasticity. In the DS 2.5 dex-HEMA hydrogels most network chains are connected to the network with two cross-links. Hydrolysis of one of these cross-links results in the loss of three elastic network chains which is reflected in the steep decrease of G' in Figure 9. The slowdown in the degradation only became apparent at the very end of the degradation since the majority of the network was already degraded by then. The initial plateau observed in the degradation profile of DS 2.5 dex-HEMA hydrogels would indicate that no hydrolysis occurred during the first 3 days. This is impossible, and the large initial swelling of the gels 1 day after their preparation probably caused the effect. In general, it could be concluded that the rheologically observable degradation kinetics of dex-HEMA hydrogels are determined by the amount of elastic cross-links present on the dex-HEMA molecules within the network. In dex-MA/dextranase hydrogels, on the other hand, it is the distance between the cross-links on a dex-MA molecule that determines the rheologically observable degradation kinetics.

As the beginning of zone III in Figure 9b (after 16 days) marks the period when the hydrolysis starts of the cross-links on dex-HEMA chains with only one cross-link left, the release of dextran from the network should start there. This lag time agreed well with t_{lag2} observed

in the release profile in Figure 8. For the DS 3.0 dex-HEMA hydrogels only a very short release lag time was observed. Because of the low DS, a part of the molecules is linked to the network with a single cross-link. Consequently, these molecules will be released early in the degradation.

3. Characterization of the Degradation through Swelling. As for dex-MA/dextranase hydrogels, the swelling behavior of dex-HEMA hydrogels was followed during degradation. From Figure 9a it is clear that a totally different behavior was found for dex-HEMA hydrogels. After an initial swelling toward equilibrium all studied dex-HEMA hydrogels continued to swell during degradation. This was partly to be expected since upon hydrolysis of a cross-link the chains that were connected to that cross-link remained intact. As illustrated in the right part of Figure 7, hydrolysis of a cross-link on a dex-HEMA molecule results in a longer network chain between the two remaining cross-links. These longer chains can expand and thereby allow the hydrogels to swell. A more detailed look at the swelling profiles of dex-HEMA hydrogels shows that the swelling in DS 8.0 hydrogels was slowed down in the beginning of the degradation although a large decrease in G' was observed. As for dex-MA/dextranase hydrogels, this could be explained by the high functionality of the cross-links. Early in the degradation, the majority of the network chains are short (due to the high DS), and as for dex-MA/dextranase hydrogels, the number of cross-links remains constant. Both seriously limit the expansion possibilities of the network. However, in contrast to dex-MA/dextranase hydrogels, this situation is not valid throughout the degradation. The right part of Figure 7 illustrates that because of the different degradation mechanism, the functionality of the cross-links in dex-HEMA hydrogels decreases much faster than that of dex-MA/dextranase hydrogels. Therefore, only a short delay in the swelling of DS 8.0 dex-HEMA hydrogels was observed.

It could be concluded that because the network chains of dex-HEMA hydrogels are not cut upon hydrolysis of a cross-link, the degradation was rather well reflected in the swelling behavior of the hydrogels. However, when the cross-links have a high functionality, the swelling is retarded compared to the rheologically observed degradation.

Conclusions

To characterize the influence of the degradation mechanism on the mechanical and swelling properties of hydrogels, two types of hydrogels were studied: dex-MA/dextranase hydrogels that degrade through hydrolysis of the polymer backbone and dex-HEMA hydrogels that degrade through hydrolysis of the cross-link. It was also evaluated to what extent the analytical data on the degradation products of the hydrogels could be compared with the rheological and swelling data of both types of hydrogels.

The rheologically observed degradation profiles (G' as a function of time) of dex-MA/dextranase hydrogels depended on the DS of the dex-MA. This was in agreement with the two-substrate model defined by Franssen et al. (1999). In contrast to the constant dextranase activity, as deducted from the release rate of degradation products, the rheologically observed degradation rates (decrease rate of G') slowed down as degradation proceeded. This could be explained by the dangling ends, created upon enzymatic hydrolysis of a network chain. Although these dangling ends could be further degraded by the enzyme, this cannot be measured rheologically because they are not elastic. Therefore, to qualitatively link the enzymatic kinetics to the rheologically observed kinetics, the two-substrate model had to be extended with a competitive inhibitor, namely the dangling ends. As long as no substrate limitations and/or nondegradable parts of the network occurred, the enzymatic hydrolysis of dex-MA/dextranase hydrogels resulted in an apparent first order decrease of G'.

While the hydrolysis of the carbonate ester in dex-HEMA is known to be a pseudo-first-order process, these kinetics were not reflected in the rheological degradation profiles. These profiles could be divided in time regions with a different G' degradation rate. It was shown that the change in the amount of elastic chains, upon hydrolysis of a cross-link on a dex-HEMA molecule, depended on the amount of cross-links present on that molecule. As this amount decreased during degradation, different degradation rates could be rheologically observed. Also, a good correlation was found between the time at which, according to the rheological data, the hydrolysis of the last cross-link present on a dex-HEMA molecule in the hydrogel started and the lag time observed for dextran release from these hydrogels.

In addition to the difference in rheological degradation kinetics observed for dex-MA/dextranase and dex-HEMA hydrogels, a different swelling behavior was also observed. Dex-MA/dextranase hydrogels hardly swelled during degradation. It was shown that due to the high functionality of the cross-links the cross-link density did not change upon degradation, preventing an expansion of the network. Dex-HEMA hydrogels on the contrary swelled significantly because hydrolysis of the cross-links resulted in longer network chains, allowing the network to expand. Moreover, the functionality of the cross-links in dex-HEMA hydrogels decreased much faster than in dex-MA/dextranase hydrogels. It was concluded that only in the case of hydrogels degraded through hydrolysis of the cross-links could swelling be used to characterize the degradation.

In general, this study showed that no straightforward answer exists to the question of what method (rheology, swelling, or determination of the degradation products) is best used to characterize the degradation of hydrogels. The success of the different methods depended on the degradation mechanism and the composition of a specific type of hydrogel. The method of choice will therefore be determined by the previewed application and the relative importance of either swelling, chemical composition, or mechanical properties of the hydrogels. All three methods are in fact complementary, and knowing the result from one analysis should allow explaining that of another. However, due to the complex nature of the polymer networks in hydrogels, the results of a specific analysis cannot be easily used to predict the results of another method. Moreover, for a correct interpretation of the results one should be aware of what the network of the hydrogel looks like.

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