# Degradation and Release Behavior of Dextran-Based Hydrogels

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ABSTRACT: Dextran hydrogels were prepared by radical polymerization of aqueous solutions of glycidyl methacrylate-derivatized dextran (dex-MA), hydroxyethyl methacrylate-derivatized dextran (dex-HEMA), and HEMA−oligolactate-derivatized dextran (dex-lactateHEMA), using potassium peroxydisulfate and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) as the initiating system. Dex-MA hydrogels only degraded under extreme conditions (100 °C, pH 1−3), whereas hydrogels derived from dex-HEMA or dex-lactateHEMA degraded fully at pH 7.2 and 37 °C, due to hydrolysis of the lactate and/or carbonate esters in the cross-links. The degradation time of these gels can be tailored from 2 days to more than 2 months by varying the nature of the spacer, the degree of substitution[1] of dextran (DS), and the initial water content of the hydrogels. The release kinetics of a model protein, Immunoglobulin G, from dex-(lactate)HEMA hydrogels were investigated and shown to be dependent on both the DS and the initial water content of the gel. Under certain conditions zero-order release was observed over a period of 10 days.

## Introduction

Hydrogels are gaining more attention as drug delivery systems, especially for the controlled release of pharmaceutically active peptides and proteins.[2−5] However, many hydrogel systems (e.g., poly(2-hydroxyethyl methacrylate),[6] poly(vinyl alcohol),[7] and poly(ethylene glycol)[8]) are not degradable under physiological conditions. Degradation of the hydrogel matrix not only circumvents removal of the empty device but it can also be used to modulate the release of an encapsulated protein from the hydrogel. Hydrolytically degradable hydrogels based on poly(ethylene glycol),[9,10] or poly(*N*-(2-hydroxypropyl)-methacrylamide)[11,12] have been reported before.

Previously, we reported on the use of dextran hydrogels for the release of proteins.[13] These hydrogels were obtained by radical polymerization of aqueous solutions of methacrylated dextrans. It was shown that the release of proteins from nondegrading highly hydrated hydrogels, derived from glycidyl methacrylate-derivatized dextran (dex-MA), can be effectively described by the free volume theory. On the other hand, the release of proteins from gels with a low hydration level was marginal, indicating that the hydrodynamic diameter of the protein was larger than the hydrogel mesh size.[13] The release of Immunoglobulin G (IgG) from these gels could be manipulated by coencapsulation of the dextran-degrading enzyme dextranase, resulting in delayed release profiles.[14] The release delay time depended on the degradation rate of the gels, which in turn was proportional to the concentration of dextranase in the gel. Manipulation of the degradation behavior of hydrogels is therefore an attractive method for tailoring release profiles of entrapped proteins.

An alternative approach toward degradable dextran hydrogels, avoiding the use of dextranase, is incorporation of hydrolytically labile spacers between dextran and the polymerized methacryloyl groups.

In the present article, we report the degradation of hydrogels made from methacrylated dextrans with different spacers between the methacryloyl group and dextran (dex-MA,[15,16] hydroxyethyl methacrylate-derivatized dextran (dex-HEMA),[17] and HEMA−oligolactate-derivatized dextran (dex-lactateHEMA);[17] Chart 1). Firstly, the relation between the network structure of the hydrogel and its degradation behavior was studied. Secondly, the influence of degradation of the hydrogel matrix on the release kinetics of the model protein IgG from degrading dex-(lactate)HEMA hydrogels was investigated.

## Materials and Methods

**1. Materials.** Dextran (from *Leuconostoc mesenteroides*, T40, $M_n$ = 15 000, $M_w$ = 32 500, as determined by GPC analysis) and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) were obtained from Fluka Chemie AG, Buchs, Switzerland. Potassium peroxydisulfate (KPS), sodium azide, and the phosphate buffer components were obtained from Merck, Darmstadt, Germany. Immunoglobulin G (IgG, fraction II) was purchased from ICN Biomedicals BV, Zoetermeer, The Netherlands. Water was purified by reverse osmosis. Bradford protein assay (Bio-Rad protein assay concentrate) was obtained from Bio-Rad Laboratories, Veenendaal, The Netherlands. All chemicals used were analytical grade. The phosphate-buffered saline (10 mM, 0.9% NaCl, pH 7.2, 0.02% $NaN_3$, abbreviated as PBS) was deoxygenated before use by bubbling nitrogen gas through it.

**2. Methods. GPC of Degraded Samples.** The molecular weights and molecular weight distributions of dex-MA and the fragments obtained after dissolution of dex-MA hydrogels (100 °C, pH 1−3) were determined by gel permeation chromatography (GPC) with a system consisting of a Model 510 HPLC pump, a Model 410 differential refractometer (both from Waters Associates Inc., Milford, MA), and three thermostated (35 °C) Shodex KW series columns (OH pack KB 800P 8 mm × 300 mm, precolumn; OH pack KB 802 6 mm × 50 mm, exclusion limit $4 \times 10^3$; OH pack KB 80M 8 mm × 300 mm, exclusion limit $2 \times 10^7$; Showa Denko, Tokyo, Japan). Prior to analysis, the solutions containing the dissolved dex-MA hydrogels were adjusted to pH 7 with sodium hydroxide and filtered over a 0.45 $\mu$m filter. Degassed 10 mM NaCl in water was used as the mobile phase.[18] The flow rate was 1.0 mL/min. The columns were calibrated with dextran standards of

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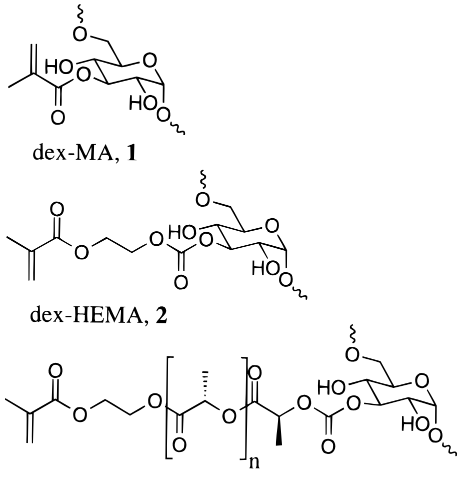
**Chart 1. Chemical Structure of Glycidyl Methacrylate-Derivatized Dextran (dexMA, 1), Hydroxyethyl Methacrylate-Derivatized Dextran (dex-HEMA, 2), and HEMA−Oligolactate-Derivatized Dextran (dex-lactateHEMA, 3)**


dex-MA, **1**

dex-HEMA, **2**

dex-lactateHEMA, **3**

known molecular weight with narrow molecular weight distribution (Fluka Chemie AG). The chromatograms were analyzed with Millennium 2010 V. 2.15 software (Waters Associates Inc.).

**Synthesis of Methacrylated Dextrans.** Dex-MA degree of substitution (DS) (4 and 11), dex-HEMA (DS 4, 9, and 13), and dex-(lactate)HEMA (DS 3, 6, and 10; average length of lactate graft is 3 monomer units) were synthesized from dextran T40 in dimethyl sulfoxide in the presence of 4-(*N*,*N*-dimethylamino)pyridine as described in detail previously.[15,17] For dex-MA, glycidyl methacrylate was used as a reagent, whereas for dex-HEMA, HEMA−carbonylimidazole (synthesized from HEMA and carbonyldiimidazole in dry tetrahydrofuran) was used to derivatize dextran. For dex-lactateHEMA, L-lactide was grafted onto HEMA, followed by activation with carbonyldiimidazole and subsequent coupling to dextran. The abbreviation dex-(lactate)HEMA is used to refer to both dex-HEMA and dex-lactateHEMA. The degree of substitution of the obtained materials was determined with $^1$H NMR in $^2H_2O$ (dex-MA and dex-HEMA) or $^2H_2O$/DMSO (dex-lactateHEMA).

**Preparation of Dextran Hydrogels (General Procedure).** Hydrogels (initial weight 1.5 g) were obtained by free radical polymerization of aqueous solutions of methacrylated dextran according to the following general procedure.

Methacrylated dextran (150 mg, corresponding to a final concentration of 10% w/w) was dissolved in 1140 $\mu$L of PBS. To this solution 135 $\mu$L of KPS in PBS (50 mg/mL; 17 $\mu$mol of KPS/g of gel) was added and mixed well. Subsequently, TEMED (75 $\mu$L; 20% (v/v) in PBS, pH adjusted to 7; 67 $\mu$mol/g of gel) was added. The resulting solution was quickly transferred into a 2 mL Eppendorf cup and incubated for 1 h at room temperature, yielding a hydrogel with an initial water content of about 90% (w/w) after polymerization. For gels with a lower water content the amounts of methacrylated dextran and buffer were adjusted correspondingly. A schematic representation of a dextran-based hydrogel is given in Figure 1.

**In Vitro Degradation of Hydrogels (General Procedure).** After polymerization, the gels were removed from the cups, cut into a cylindrical shape (length 2 cm, radius 0.46 cm) and accurately weighed ($W_0$, about 1.2 g). The weighed hydrogels were placed in vials containing 30 mL of the appropriate buffer, which were placed in a water bath at 37 or 100 °C. At regular time intervals, the weight of the gels was determined and used to calculate the swelling ratio (defined as $W_t/W_0$, in which $W_t$ is the weight of the gel at time t and $W_0$ is the initial weight of the gel). The hydrogel degradation time is defined as the time needed for complete degradation ($W_t = 0$). The swelling profile of a gel was determined in triplicate, except for the gels degraded at 100 °C.

**Dex-MA Hydrogels.** Prior to degradation, the dex-MA gels were extracted and equilibrated in demineralized water at 4 °C for 24 h. The degradation of hydrogels prepared from dex-MA DS 11 (initial water content 80%) was determined in the pH range 1.4−11.3, both at 37 °C and at 100 °C. The degradation of hydrogels prepared from dex-MA DS 4 (initial water content 90%) was studied at pH 1.4, 4, 7, and 11.3, at 37 °C. Buffers used were hydrochloric acid for pH 1−2, acetate for pH 3−6, phosphate for pH 7−8, borate for pH 9, carbonate for pH 10−10.4, and sodium hydroxide for pH 11.3. Buffer concentrations were 100 mM except for pH 2 and 11.3, for which the concentration was 10 mM. The ionic strength ($\mu$) of all buffers was adjusted to 0.3 with sodium chloride. The buffer solution was replaced completely each time the weight of the gel was determined.

**Dex-(lactate)HEMA Hydrogels.** The degradation of dex-(lactate)HEMA gels was studied at 37 °C at pH 7.2 in PBS (10 mM). The gels were weighed periodically, and after each measurement 2.5 mL of the incubation medium was replaced by fresh buffer.

**Determination of the Cross-Link Density ($M_c$).** For hydrogel materials, the molecular weight between the cross-links ($M_c$) can be calculated with the model of Flory and Rehner,[19] modified by Peppas and Merrill[20] for gels in which the cross-links are introduced in solution (eq 1):

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{(\bar{v}/V_1)[\ln(1 - \nu_{2,s}) + \nu_{2,s} + \chi_1(\nu_{2,s})^2]}{\nu_{2,r}[(\nu_{2,s}/\nu_{2,r})^{1/3} - 0.5(\nu_{2,s}/\nu_{2,r})]} \qquad (1)$$

where $M_n$ is the number average molecular weight of dextran ($16 \times 10^3$ Da), $\nu$ is the partial specific volume of dextran (0.62 cm$^3$/g),[21] $V_1$ is the molar volume of water (18 cm$^3$/g), $\chi_1$ is the Flory polymer-solvent interaction parameter (0.473 for dextran/water),[22] $\nu_{2,r}$ is the polymer fraction of the hydrogel in a relaxed state (i.e. directly after gel formation), and $\nu_{2,s}$ is the polymer fraction at equilibrium swelling. $\nu_{2,r}$ and $\nu_{2,s}$ were calculated from the weight of the gels before exposure to the buffer solution and after equilibrium swelling, respectively, assuming volume additivity of water and dextran.

**Release of IgG from Dextran Hydrogels.** Dex-lactateHEMA hydrogels containing approximately 3.5 mg of IgG per gram of gel were prepared according to the general procedure described above. To incorporate IgG, 100 $\mu$L of the PBS was replaced by the same volume of a solution of IgG (50 mg/mL) in PBS. The release of IgG was determined by incubation at 37 °C in vials containing 30 mL of PBS at pH 7.2. The vials were gently shaken at 100 strokes per minute, and 2.5 mL samples were taken periodically and replaced by fresh buffer. The protein concentration was measured with the Bio-Rad protein assay.[23] Since the degradation compounds (among others oligomethacrylates) interfered with the protein assay, the release of IgG from the degrading gels was quantified until the matrix of the gels started to dissolve.

## Results and Discussion

**Swelling and Degradation Behavior of dex-MA Hydrogels.** Upon exposure to water, hydrogels reach an equilibrium swelling after a certain time. In the equilibrium state, the swelling and the elastic retractive forces are in balance.[24] The distance between two consecutive cross-links in a hydrogel, when expressed as molecular weight, is $M_c$, the number average molecular weight between the cross-links (eq 1; Figure 1). When part of the cross-links is hydrolyzed, $M_c$ increases, which results in an increase in swelling of the hydrogel.

The influence of pH on the swelling behavior of dex-MA hydrogels was studied in gels with two different cross-link densities. A dex-MA hydrogel with DS 4 and an initial water content of 90% ($M_c$ = 4100 Da; eq 1) was compared with a dex-MA hydrogel with DS 11 and an initial water content of 80% ($M_c$ = 780 Da). Figure

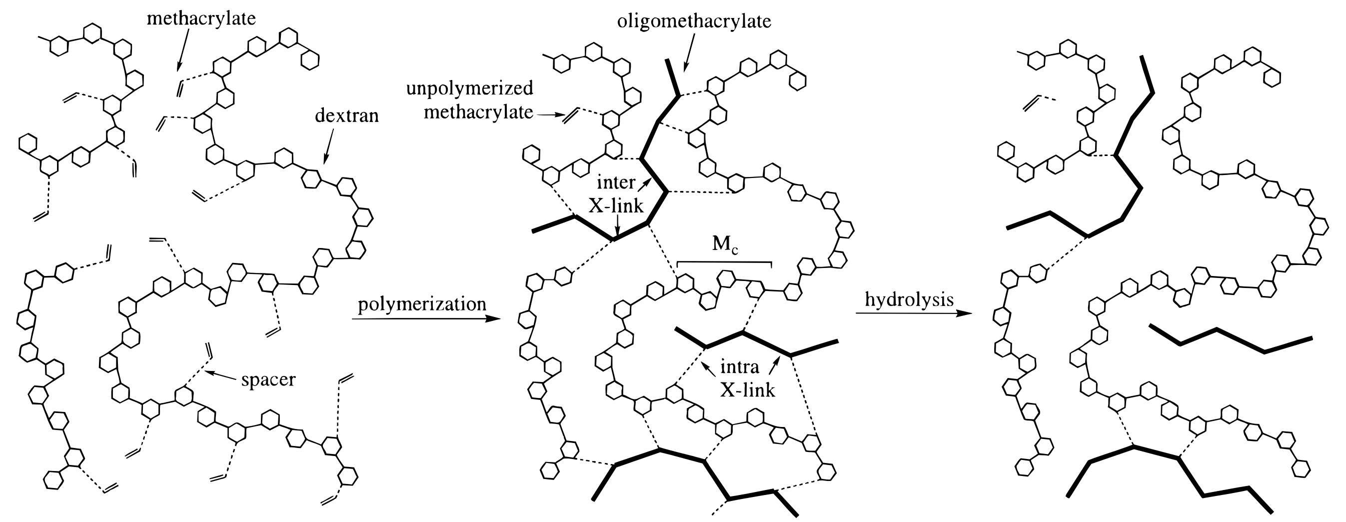


**Figure 1.** Schematic representation of the formation of a hydrogel, obtained by polymerization of methacrylated dextran, and subsequent hydrolysis. The dashed lines represents the spacer between the methacryloyl group and dextran, which is an ethylene glycol group with and without an oligolactate graft for dex-lactateHEMA and dex-HEMA, respectively; the spacer is absent in dex-MA. The inter- and intramolecular cross-links (X-link) are also indicated, as well as the molecular weight between the cross-links, $M_c$.

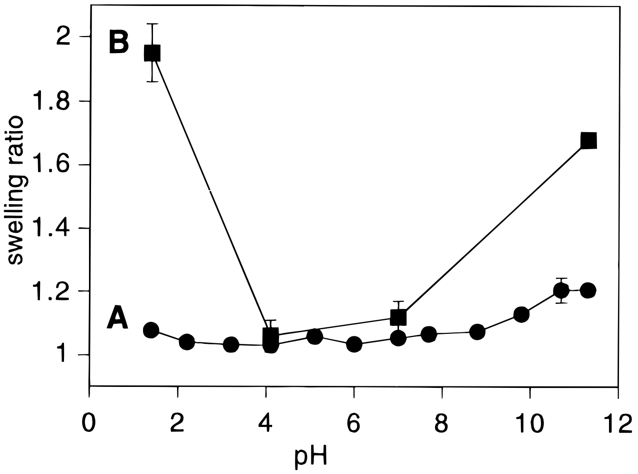


**Figure 2.** Swelling ratio of dex-MA hydrogels after 5 months of incubation at 37 °C as a function of the pH: (A) DS 11 and 80% water; (B) DS 4 with 90% water (average ± SD, $n = 3$).

2 shows the swelling ratio of the gels after 5 months at 37 °C at various pH values. The gel with the high cross-link density (DS 11, 80% water) reached a swelling ratio of about 1.20 (corresponding with an $M_c$ of 1800 Da) in 5 months at pH 11.3, whereas at pH 1.4 the swelling ratio increased to 1.08 (corresponding with an $M_c$ of 1400 Da). On the other hand, the gels with the lower cross-link density showed a much higher swelling ratio of 1.95 (pH 1.4) and 1.65 (pH 11.3), corresponding with $M_c$ values of 6100 and 5500 Da, respectively. At physiological pH, neither gel showed a detectable increase in swelling, indicating that no significant hydrolysis of the polymerized methacrylate esters occurred. It has been reported that hydrogels derived from acrylated poly(ethylene glycol) were also stable at pH 7.2 and 37 °C.[10]

In a previous study,[25] the half-lives of dex-MA at 37 °C and at various pH values were determined. At pH 1.4 its half-life is about a year, whereas at pH 10.4 this value is about 30 min. Considering that a dex-MA hydrogel at the latter pH still had not dissolved after 5 months (corresponding with about 7000 half-lives of dex-MA), it is clear that the methacrylate esters in dex-MA hydrogels hydrolyze much slower than the esters in dex-MA, especially under alkaline conditions. An explanation might be that the polymerized methacrylate ester is less sensitive toward alkaline hydrolysis than the corresponding unpolymerized ester. In the same study,[25] we indeed demonstrated that the alkaline hydrolysis of methyl isobutyrate, representing the repeating unit in poly(methacrylate), proceeded 18 times more slowly than the hydrolysis of methyl methacrylate. However, this alone can not explain the resistance of the methacrylate esters in dex-MA hydrogels toward hydrolysis. Two other factors may contribute to the hydrolytic stability of polymerized methacrylate esters. Firstly, at a high pH, a hydrolyzed and deprotonated methacrylic acid repels the approach of a negatively charged hydroxyl ion, which results in slower degradation of neighboring esters, as was observed for the hydrolysis of poly(methyl acrylate).[26] A similar effect plays a role in the alkaline hydrolysis of esters of diacids (such as dimethyl succinate) in which the second ester is hydrolyzed much more slowly than the first ester.[26] Secondly, another factor that may contribute to the stability of the polymerized methacryloyl groups is shielding of the methacrylic esters by the bulky methyl groups in the backbone of the polymer.[27]

Hydrogels of dex-MA DS 11 with 80% initial water content were also incubated at 100 °C to accelerate the degradation. At pH 11.3 the gel did not dissolve, even after 100 days. This again demonstrates that, compared to the case for dex-MA, alkaline hydrolysis of polymerized methacrylate esters is retarded. Under acidic circumstances the gels did dissolve. The dissolution times were around 1 day, 5 days, and 40 days at pH 1, 2, and 3, respectively. However, under these acidic conditions dextran is degraded by hydrolysis of the glycosidic linkage (GPC analysis; results not shown). Therefore, no conclusion can be drawn concerning the stability of the methacrylate esters in a dextran hydrogel at low pH.

**Swelling and Degradation Behavior of Dex-(lactate)HEMA Hydrogels.** Figure 3 shows the swelling behavior of a dex-MA hydrogel, a dex-HEMA hydrogel, and a dex-lactateHEMA hydrogel, all with an initial water content of 90%. The dex-MA (DS 4) hydrogel reached an equilibrium swelling within 3 days;

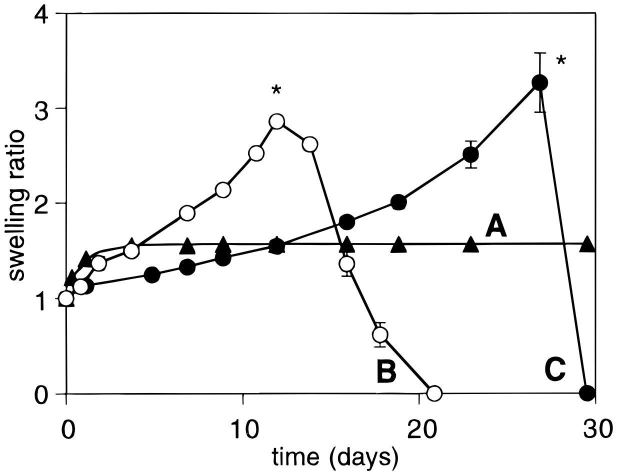


**Figure 3.** Influence of spacer on the swelling behavior of dextran hydrogels with initial water content 90%: (A) dex-MA DS 4; (B) dex-lactateHEMA DS 10; (C) dex-HEMA DS 9 (average ± SD, $n = 3$). The asterisk indicates the time of maximum swelling, which is the transition from the swelling phase to the dissolution phase of the gel.

**Table 1. GPC Analysis of Dextran and Dex-(lactate)HEMA Hydrogels after Incubation at pH 11.3 and 37 °C for 48 h**

| compound | $M_n$ (kDa) | $M_w$ (kDa) |
|---|---|---|
| dextran T40 before incubation (Fluka) | 21 | 40 |
| dextran T40 after incubation | 21 | 41 |
| degraded dex-HEMA gel (DS 13, 90%) | 19 | 34 |
| degraded dex-lactateHEMA gel (DS 10, 90%) | 21 | 35 |

thereafter, the weight of the gel remained constant, again demonstrating that no significant hydrolysis of ester groups in the cross-links occurred. On the other hand, both the dex-HEMA and dex-lactateHEMA gels showed a progressive swelling in time, followed by a dissolution phase. Obviously, due to hydrolysis of esters, the molecular weight between the cross-links ($M_c$) increases, resulting in an increased swelling. When the maximum swelling ratio was reached, the $M_c$ of both the dex-HEMA gel and the dex-lactateHEMA gel is about 7000 Da. The $M_c$ determined via swelling measurements, however, results in an underestimation of the actual molecular weight between the cross-links.[13] This might indicate that the dextran chains only bear about one cross-link per chain ($M_n$ of dextran is 15 kDa) at the point the gel starts to disintegrate and soluble products are formed. This results in a decrease in gel weight and finally in a complete dissolution of the hydrogel. Interestingly, this dissolution phase is much longer for dex-lactateHEMA hydrogels than for dex-HEMA gels, especially at high cross-link density. No satisfying explanation is available for this difference.

Compared with a dex-MA hydrogel, the dex-HEMA gel has a hydrolyzable carbonate ester in its cross-links (Chart 1), which is apparently labile under physiological conditions. The dex-lactateHEMA hydrogel contains, apart from a carbonate ester, also lactate esters in the cross-links (Chart 1), which are known to degrade under physiological conditions.[28] A dex-HEMA gel (DS 13) and a dex-lactateHEMA gel (DS 10) with an initial water content of 90% were incubated at pH 11.3 for 48 h to accelerate the degradation. Upon dissolution of the gels, the formed products were analyzed with GPC. Table 1 shows that the molecular weight of dextran obtained after degradation of dex-(lactate)HEMA was not significantly different from that of dextran used to prepare the materials, indicating that these gels indeed degrade by hydrolysis of the labile bonds in their cross-links.

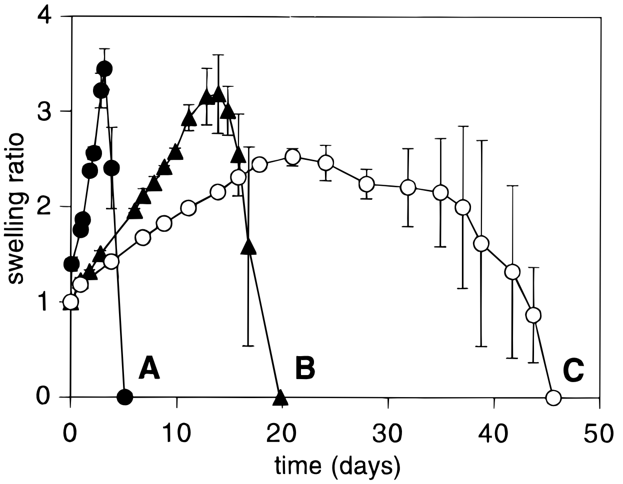


**Figure 4.** Swelling behavior and degradation time of dex-lactateHEMA hydrogels with an initial water content of 80%: (A) DS 3; (B) DS 6, (C) DS 10 (average ± SD, $n = 3$).

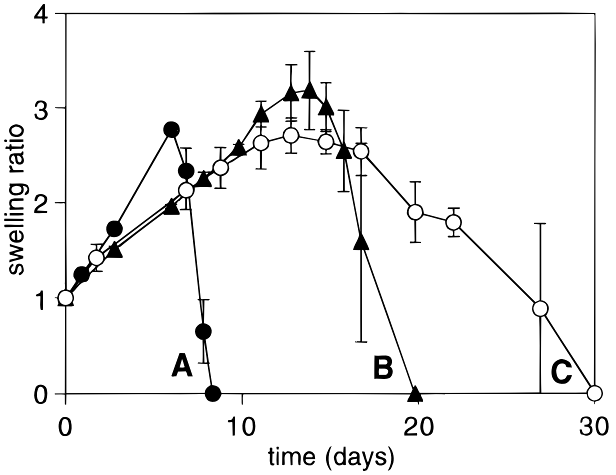


**Figure 5.** Swelling behavior and degradation time of dex-lactateHEMA hydrogels with DS 6: initial water content 90% (A), 80% (B), and 70% (C) (average ± SD, $n = 3$).

Figures 4 and 5 show the swelling behavior of dex-lactateHEMA gels as a function of the DS at a fixed initial water content (80%) and as a function of the initial water content at a fixed DS (6), respectively. It appears that the degradation time increased with increasing DS (Figure 4) and decreased with increasing initial water content (Figure 5).

The degradation times of dex-HEMA gels were also studied as a function of DS and initial water content (results not shown). Figure 6 summarizes the degradation times of all dex-(lactate)HEMA gels investigated. This figure shows that for both gel systems the degradation time increased with an increasing DS. It is obvious that an increasing DS at a fixed initial water content results in a network with a higher cross-link density. To dissolve the network, more cross-links have to be hydrolyzed, which requires more time.

Figure 6 also shows that, at a fixed DS, the degradation time increased with a decreasing initial water content. In a previous study by our group,[13,29] it was shown that a decreasing initial water content resulted in a decreasing $M_c$. This was attributed to an increased contribution of intermolecular cross-linking at higher dextran concentrations. Again, an increasing cross-link

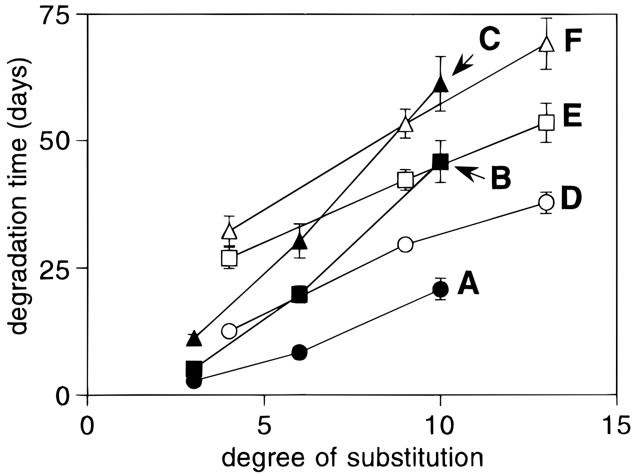


**Figure 6.** Degradation time of dex-HEMA (open symbols) and dex-lactateHEMA hydrogels (closed symbols) as a function of DS at various initial water contents; dex-lactateHEMA 90% (A), 80% (B), and 70% (C); dex-HEMA 90% (D), 80% (E), and 70% (F) (average ± SD, $n = 3$).

density (decreasing $M_c$) requires a longer time to dissolve the network.

At DS < 10, the dex-lactateHEMA gels degraded faster than the dex-HEMA gels with the same initial water content. This indicates that the lactate esters in dex-lactateHEMA are more easily hydrolyzed than the carbonate esters. However, when the initial water content of dex-lactateHEMA gels with DS 10 is 80% or 70%, a degradation time was found which would be expected for dex-HEMA hydrogels of that composition. This indicates that in dex-lactateHEMA hydrogels with a high DS (10) and a low initial water content (<80%) the carbonate ester is the most labile bond in the cross-link. An explanation might be the association of the hydrophobic lactate spacers in the gels with a lower water content and high DS, retarding the hydrolysis of the lactate esters. This is supported by two other observations. Firstly, before polymerization, solutions of dex-lactateHEMA DS 10 with 80 and 70% water content showed an enormous increase in viscosity. Secondly, dex-lactateHEMA gels were opaque, in contrast with dex-MA and dex-HEMA hydrogels, which were transparant, indicating that in dex-lactateHEMA gels phase separation occurred.

Previously, we studied the hydrolysis of dex-HEMA in solution, and determined the pseudo-first-order reaction rate constant $k_{obs}$ at several pH values.[25] $k_{obs}$ can be described by the general rate constant equation (eq 2), where $k_0$ is the first-order rate constant for degradation in water only, and $k_H$, $k_{OH}$, and $k_b$ are second-order rate constants for degradation catalyzed by protons, hydroxyl ions, and buffer ions, respectively. At pH 7, $k_0$, $k_H[H^+]$, and $k_b$ are negligible and $k_{obs}$ is approximately equal to $k_{OH}[OH^-]$.

$$k_{obs} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_b[buffer] \quad (2)$$

**Table 2. Time When Dex-(lactate)HEMA Hydrogels Start To Dissolve and the Calculated $k_{obs}$ of the Hydrolysis of the Cross-links in the Gels**

| | start of dissolution[a] (days) | | | calculated $k_{obs}$[b] ($10^{-6}$ s$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 90% | 80% | 70% | 90% | 80% | 70% |
| | | | Dex-HEMA | | | |
| DS 4 | 9 | 23 | 27 | 1.7 | 0.69 | 0.59 |
| DS 9 | 27 | 37 | 44 | 0.94 | 0.68 | 0.58 |
| DS 13 | 30 | 48 | 51 | 0.98 | 0.61 | 0.58 |
| | | | Dex-lactateHEMA | | | |
| DS 3 | 1.8 | 3.1 | 5.1 | 8.8 | 5.2 | 3.1 |
| DS 6 | 6 | 14 | 14 | 4.3 | 1.8 | 1.8 |
| DS 10 | 12 | 21 | 44 | 2.4 | 1.4 | 0.68 |

[a] Taken as the time when maximum swelling is reached (Figure 3). [b] $k_{obs}$ = [ln DS(0)/DS($t$)]/degradation time, where DS(0) is the DS of the starting material and DS($t$) is set at 1 (one cross-link per dextran chain of 15 kDa (≈100 glucopyranose residues) corresponds with a DS of about 1).

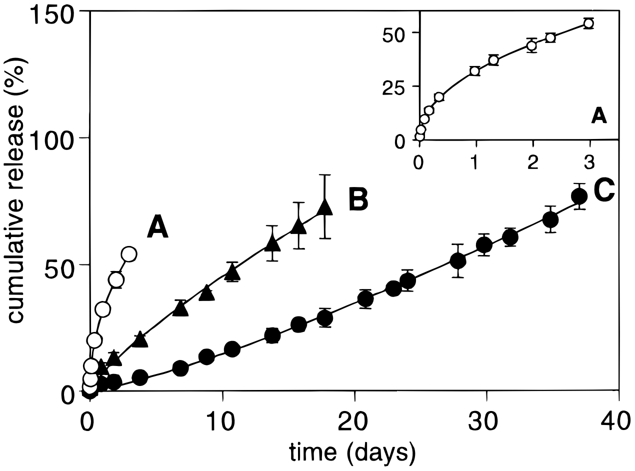


**Figure 7.** Cumulative release of IgG in time from dex-lactateHEMA hydrogels (DS 10) with initial water content 90% (diffusional exponent $n = 0.46$) (A), 80% ($n = 0.79$) (B), and 70% ($n = 1.24$) (C) (average ± SD, $n = 3$).

The hydrolysis rate of the cross-links in dex-HEMA and dex-lactateHEMA after polymerization into hydrogels can also be calculated assuming pseudo-first-order kinetics. As mentioned before, at the time that the gel starts to dissolve (the time at which the swelling ratio of the gels reaches a maximum, Figure 3B and C), the dextran chains most likely bear one cross-link per chain. Since the amount of cross-links at the start of the degradation can be derived from the degree of substitution of the methacrylated dextran, the pseudo-first-order reaction rate constant $k_{obs}$ for the hydrolysis of the cross-links in dex-HEMA and dex-lactateHEMA hydrogels can be calculated.

Table 2 summarizes the calculated values of $k_{obs}$ for each gel composition. For the dex-HEMA hydrogels the values vary between $0.6 \times 10^{-6}$ and $1.7 \times 10^{-6}$ s$^{-1}$ and correspond well with the $k_{obs}$ of dex-HEMA in solution, which is $1.6 \times 10^{-6}$ s$^{-1}$ at pH 7.2 and 37 °C. We showed that at pH 7 degradation of dex-HEMA takes place primarily by hydrolysis of the carbonate ester.[25] This means that dex-HEMA hydrogels also degrade via hydrolysis of the carbonate ester in the cross-links. Since hydrogels derived from dex-lactateHEMA DS < 10 were shown to degrade much faster than dex-HEMA gels, the $k_{obs}$ values calculated for the former gels are much higher (between $1.8 \times 10^{-6}$ and $8.8 \times 10^{-6}$ s$^{-1}$). This again indicates that, in dex-lactateHEMA gels with low DS and a high water content, the lactate ester is more easily cleaved than the carbonate ester.

**Protein Release from Degrading Dex-(lactate)-HEMA Hydrogels.** The release of a model protein, Immunoglobulin G (IgG), from degrading dex-lactate-HEMA hydrogels with varying DS values and initial water contents was investigated. Representative release profiles are shown in Figures 7 and 8. The release rate of IgG from gels with a DS of 10 decreased with a decreasing initial water content (Figure 7). The release

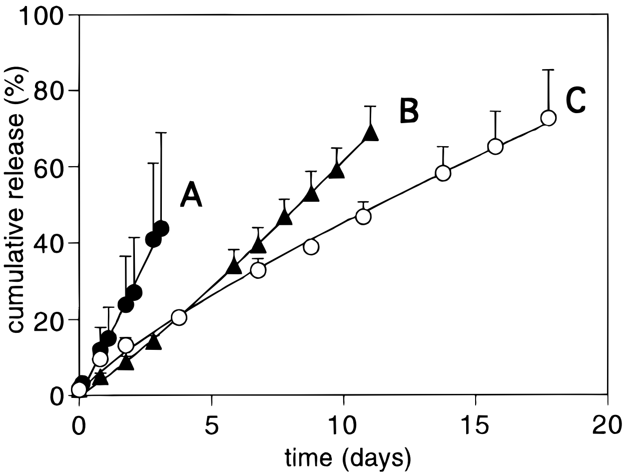


**Figure 8.** Cumulative release of IgG in time from dex-lactateHEMA (80% initial water content) with DS 3 (diffusional exponent $n = 1.03$) (A), DS 6 ($n = 1.12$) (B), and DS 10 ($n = 0.80$) (C) (average ± SD, $n = 3$).

rate of IgG from gels with the same initial water content of 80% also decreased with an increasing DS (Figure 8).

The release profiles were recorded in the swelling phase of the hydrogel degradation process and not during the dissolution phase (Figures 3−5). The profiles can therefore be analyzed using the semiempirical power law equation, as suggested by Peppas[30,31] for the release of drugs from swellable matrices (eq 3)

$$\frac{M_t}{M_\infty} = kt^n \tag{3}$$

in which $M_t/M_\infty$ is the fractional release of the drug in time $t$, $k$ is a constant, characteristic for the drug−matrix sytem, and $n$ is the diffusional exponent, characteristic for the release mechanism. For diffusion-controlled release, $n$ equals 0.5 (Fickian diffusion), whereas for hydrogels in which the water penetration (swelling) is controlled by polymer relaxation (in this study hydrolysis of the cross-links), $n$ has a value between 0.5 and 1.

In degrading hydrogels, two opposing effects occur. Firstly, during swelling of hydrogels, the size of the hydrogel increases and the concentration of the protein in the gel decreases. Both effects result in a decrease in protein concentration gradient, which is the actual driving force for release, and therefore in a decreasing release rate.

Secondly, due to the swelling, the volume fraction of water in the gel increases. It was shown for highly hydrated dextran gels that the diffusion process of proteins can be described with free volume theory.[13] This theory relates the diffusion coefficient of the solute to the size of the solute and the hydration of the hydrogel (eq 4)

$$\ln\left(\frac{D_m}{D_0}\right) = \ln(\Phi) - \left(\frac{kr^2}{V_{f,water}}\right)\left(\frac{1}{H} - 1\right) \tag{4}$$

where $D_m$ is the diffusion coefficient of the solute in the matrix, $D_0$ is the diffusion coefficient of the solute in water, $\Phi$ indicates the screening effect of the matrix ($\Phi = 1$ when the size of the solute is smaller than the hydrogel mesh size), $r$ is the hydrodynamic radius of the solute, $V_{f,water}$ is the free volume in water, $k$ is a constant, and $H$ is the hydration of the hydrogel (=the volume fraction of water in the gel).

It can therefore be concluded that the increasing hydration of a swelling hydrogel causes an increase in the diffusion coefficient of the protein in the hydrogel matrix, which in turn results in an increase in release rate.

**Table 3. Diffusional Exponents ($n$) for Release of IgG from Dex-lactateHEMA Hydrogels**[a]

| DS | 90% | 80% | 70% |
|---|---|---|---|
| 3 | 0.56 ± 0.02 | 1.02 ± 0.07 | 1.30 ± 0.07 |
| 6 | 0.54 ± 0.01 | 1.07 ± 0.05 | 1.17 ± 0.22 |
| 10 | 0.46 ± 0.01 | 0.71 ± 0.15 | 1.16 ± 0.08 |

[a] Average ± s.d. of three release curves per gel composition.

Table 3 shows the diffusional exponents for the different dex-lactateHEMA hydrogels investigated. The diffusional exponent is about 0.5 in gels with 90% initial water content. Obviously, the release rate increasing factor (increased diffusion coefficient) and release rate decreasing factor (decreased protein concentration gradient) counteract each other, resulting in a release which can be described by a Fickian diffusion process.

In gels with an intermediate water content (80%), a value between 0.7 and 1.0 is found for the diffusional exponent $n$, indicating that the release is controlled by the swelling (degradation) of the matrix. Obviously, the increase in protein diffusion coefficient contributes more to the final release than the release rate decreasing factor, the decrease in concentration gradient. These gels even exhibit almost constant release (zero order, $n = 1$) of IgG from gels for a period of 10 days (Figure 8B).

In gels with a relatively low initial water content (70%), $n$ is greater than 1. Due to screening effects, the initial release was marginal. However, degradation results in an increase of the mesh size in time. It can be expected that the release increases exponentially when the gel is degraded to such an extent that the mesh size has become greater than the protein diameter, resulting in a release profile as shown in Figure 7C.

## Conclusions

Methacrylated dextran can be polymerized in aqueous solution by radical polymerization with $N,N,N',N'$-tetramethylethylenediamine and potassium peroxydisulfate. Hydrogels in which the polymethacrylate esters were directly linked to the dextran chains were very stable, even under extreme conditions. However, introduction of a hydroxyethyl group with and without an oligolactate graft, between the methacryloyl groups and the dextran chains results in hydrolytically labile hydrogels, which degrade under physiological conditions. The degradation times of these hydrogels can be tailored by the nature of the spacer, the degree of substitution, and the initial water content of the hydrogel. Release of the model protein Immunoglobulin G from degrading hydrogels was dependent on the composition of the hydrogel. In certain cases zero-order release of protein was observed over a 10 day period.

## References and Notes

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