Characterization of the Network Structure of Dextran Glycidyl Methacrylate Hydrogels by Studying the Rheological and Swelling Behavior†

S. C. De Smedt,* A. Lauwers, and J. Demeester

Laboratory of General Biochemistry and Physical Pharmacy, University of Gent, Wolterslaan 16, 9000 Gent, Belgium

M. J. Van Steenbergen and W. E. Hennink

Utrecht Institute for Pharmaceutical Sciences, Department of Pharmaceutics, University of Utrecht, 3508 TB Utrecht, The Netherlands

S. P. F. M. Roefs

NIZO, Netherlands Institute for Dairy Research, 6710 BA Ede, The Netherlands

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ABSTRACT: This paper reports the results and the structural interpretation of rheological, swelling, and analytical sol fraction (w_s) measurements performed on dextran glycidyl methacrylate (dex-gma) hydrogels as a function of the dex-gma concentration (c) and the degree of gma substitution (DS). Besides the analytical determination, the sol fraction was also calculated from the elasticity of the hydrogels using a theoretical model. This model takes into account the presence of sol chains and dangling ends, the polydispersity of the dex-gma chains, the functionality of the junctions, and the nonaffinelike behavior of the dex-gma network. It assumes the absence of intramolecular cross-links and of physical entanglements. Fundamental in the discussion is the reason why the w_s values determined analytically are systematically lower than the w_s values calculated using this theoretical model. Besides possible influences from the unknown functionality of the junctions and from a nonaffinelike deformation behavior of the network, the presence of intramolecular cross-links (especially for hydrogels with a low dex-gma concentration and a high DS) may contribute to the observed differences between the measured and the calculated sol fraction. Structural information is also revealed from the influence of the DS on the elasticity if plotted against the network concentration (i.e., $(1 - w_s)c$) of the hydrogels. Clearly, a DS increase increases quantitatively the network fraction. However, for high DS values, a DS increase does not increase the number of intermolecular cross-links per unit of mass present in the network fraction. Also the abundant presence of intramolecular cross-links, especially for dex-gma hydrogels with a high DS, may contribute to this phenomenom. Contrary to the elastic properties, considering hydrogels with the same network concentration, the DS does have a definite influence on the swelling properties of the network fraction of the gels. This was attributed to the dependence of the polymer–solvent interaction parameter on the DS.

Introduction

Due to the intensive research on polymer networks, the term "gel" is used so frequently that we feel it necessary to define the meaning of the term "hydrogel".[1] In the context of this paper, it indicates a polymer network created by chemical cross-linking of an aqueous solution of glycidyl methacrylate substituted dextran polymer chains (see Figure 1). Dextran is an anhydroglucose polymer consisting mainly of α-1,6-glucosidic linkages. The term "relaxed hydrogel" is used for the dex-gma network immediately after cross-linking but before swelling. After swelling, a "swollen hydrogel" will be formed.

Although dextran hydrogels are frequently used as column packing in all kinds of chromatographic applications, pharmaceutical research has only recently begun on the potential of dextran hydrogels in the field of *controlled* drug delivery. Main topics in this field are the stabilization, targeting, and release of therapeutic active proteins enclosed in acryldextran microspheres, colon drug delivery, and nasal drug delivery from dextran hydrogels.[2–5] In these pharmaceutical formulations a key point is understanding how the diffusion process of the drug is influenced by the structure of the polymer network. The research on dex-gma hydrogels described in this paper is a follow up of our previous studies on the network structure and the diffusion of probes in hyaluronic acid solutions.[6,7] This paper represents the results of rheological and swelling experiments carried out on dex-gma hydrogels to elucidate structural features of dex-gma hydrogels.

The most important property of an elastic polymer network is its degree of cross-linking, i.e., the number density of junctions or cross-links joining the chains into a network structure which gives rise to elastic properties.[8,9] In general, the number of moles of elastic chains per unit volume (ν) and the number of moles of elastic junctions (cross-links) per unit volume of the network (μ) are used to describe the cross-link density of the polymer network. Consequently, ν and μ determine the average molecular weight between the junctions (M_c). The functionality of the junctions (f), being the number of chains leaving from one junction, determines the relation between ν and μ by the following expression:[8]

$$\mu = 2\nu/f \tag{1}$$

Mostly, rheological elasticity measurements and swelling experiments are used to estimate ν, μ, and M_c for polymer networks.[10] The obtained values are, in fact,

† This paper is dedicated to Professor Josef Schurz on the occasion of his 70th birthday.

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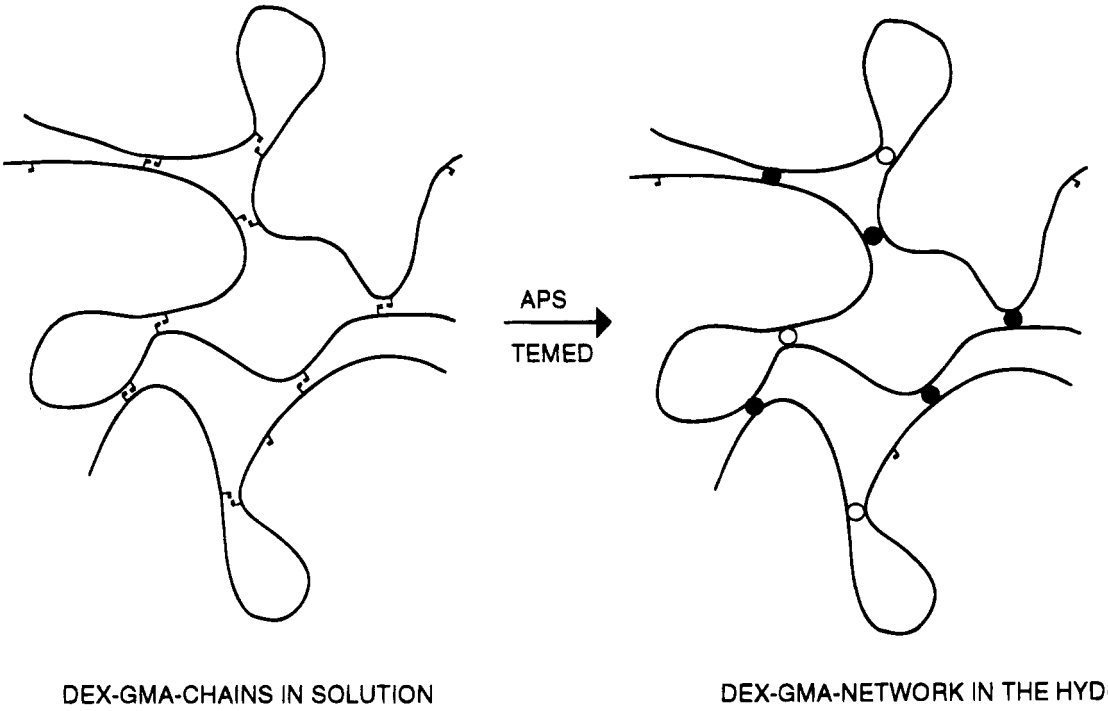

Figure 1. Schematic representation of the cross-linking reaction. Due to the radical reaction between the double bonds of the gma groups (|), inter- (●) and intramolecular (○) junctions join the dextran chains into a network.

estimates rather than accurate values, as molecular models are required to determine the information from the experiments. Such models are developed for ideal networks, i.e., networks containing only elastic chains without any defects like nonelastic dangling ends, loops and chains not attached to the network, no trapped entanglements (contributing to the elastic properties), nor heterogeneous regions.

An important consideration in the development and interpretation of theoretical molecular models is the difference between affine networks and phantom networks. In an affine network, it is assumed that the junctions of the network do not fluctuate and that they transform affinely (linearly) with the macroscopic deformation.[8,9] For an affine network it is assumed that only the network chains contribute to the decrease of entropy of the network (which gives rise to the elastic force) upon deformation. For a phantom network it is assumed that the junctions do fluctuate over time.[11] For the affine network model, the equilibrium shear modulus of the network (G_e) is given by:[8]

$$G_e = \nu RT \tag{2}$$

For the phantom network, due to the fluctuations of the junctions, G_e is lower than that of the corresponding affine network:[11]

$$G_e = (\nu - \mu)RT \tag{3}$$

Real networks are expected to show characteristics which lie somewhere between the properties of both affine and phantom models. This phenomenom was treated by Flory and Erman in the constrained junction model which allows for this intermediate behavior:[9]

$$G_e = (\nu - h\mu)RT \tag{4}$$

In eq 4 h ranges between 0 (for an affine network) and 1 (for a phantom network).

As the network structure of the dex-gma hydrogels is nonideal, the calculation of the structural network parameters from G_e was based on a molecular model which partially took into account structural imperfections.[12-15]

A key point in the explanation of the results is the sol fraction (w_s) of the dex-gma networks which is that part of the polymer molecules not attached to the infinite network. On the basis of the comparison between the w_s-value calculated from the molecular model used (w_s(G)) and the w_s-value analytically determined (w_s(ANAL)), we are able to elucidate some structural features of the dolex-gma hydrogels.

Experimental Section

Dex-gma-preparation and Characterization. Four batches of methacrylated dextran were prepared: for each batch 50 g of dextran (Fluka Chemie AG; cat. no. 31389; M_n = 22 200) was dissolved in 450 mL dimethylsulfoxide (Merck). Each batch received 10 g of 4-(dimethylamino)pyridine. Respectively 7.3, 5.5, 4.4, and 2.9 g of glycidyl methacrylate (gma; Fluka Chemie AG; cat. no. 64161) were added to the solutions corresponding with a ratio of 1 mol of gma per 6, 8, 10, and 15 mol of glucopyranosyl units. The reaction was performed at room temperature and stopped after 24 h by adding the reaction mixture drop by drop in 2000 mL of ethanol (technical grade) while stirring. The precipitate was collected by filtration and dissolved in 100 mL of double-distilled water. The solution was adjusted to pH 8 with hydrochloric acid and dialyzed against demineralized water for 48 h. The methacrylated dextran was dried by lyophilization. The degree of gma substitution or DS (i.e., the molar ratio of gma per glucopyranosyl unit) was determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) in D_2O with a Gemini 300 spectrometer (Varian).[3] The DS of the batches was respectively $^1/_{10}$, $^1/_{22}$, and $^1/_{23}$. The DS of the fourth batch was too low to determine accurately (DS < $^1/_{23}$). A random gma substitution of the dextran chains was assumed.

Preparation of the Dex-gma Hydrogels. Dex-gma hydrogels were prepared by radical reaction of aqueous dex-gma solutions (solvent: 0.2 M Na_2HPO_4, 1 mM NaEDTA, pH 8.5) as a function of the dex-gma concentration and the gma

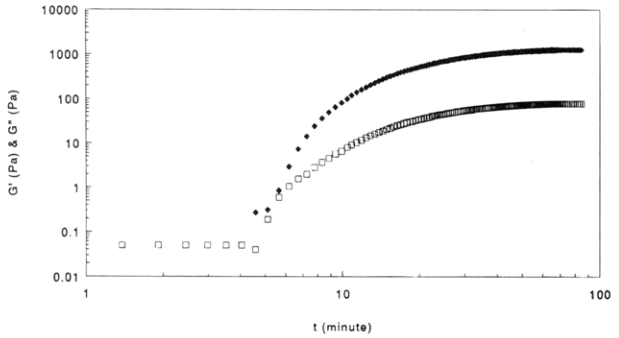

Figure 2. Change of the shear storage modulus (G') and the shear loss modulus (G'') as a function of the reaction time (t): G' (◆); G'' (□).

substitution degree. Figure 1 represents schematically the cross-linking reaction. Solution A was obtained by adding 30 μL of *N,N,N′,N′*-tetramethylethylenediamine (TEMED) to 3.0 mL of a dex-gma solution, while solution B was a mixture of 2.5 mL of a dex-gma solution and 125 μL of ammonium persulfate (APS; 80 mg/mL). Solution A (2.5 mL) was added to solution B (2.5 mL). Approximately 5 min after adding solution A to solution B (depending on the dex-gma concentration and the DS), the gelation started, and it was completely finished after roughly 90 min (see the Rheological Experiments section). From infrared measurements performed on the dex-gma hydrogels it appeared that more than 90% of the gma groups had reacted.

Rheological Experiments. The elasticity measurements were performed on a controlled-stress Haake RS100 rheometer. A plate−plate measuring system was used (diameter 35 mm); the gap between the plates was 0.8 mm (measuring temperature 20 °C). During the experiment, the gelation of the dex-gma solutions occurred between the plates of the measuring system, and consequently the rheological measurements were carried out on the relaxed dex-gma hydrogels. Since the dex-gma hydrogels remained between the measuring plates of the instrument for about 2 h, it was necessary to use a solvent trap which prevented evaporation of the solvent.

On each dex-gma hydrogel three types of rheological experiments were performed:

Type a. To determine the end of gelation, the reaction was monitored by measuring the shear storage modulus (G'). G' was measured as a function of the gelation time (frequency 0.7 Hz). The stress used in these experiments was as low as possible in order to minimize the influence of the deformation during the experiment on the formation of the dex-gma hydrogel. Figure 2 represents the typical behavior of G' during the gelation reaction. Three time regions can be distinguished. During the induction period G' is too small to be measured. During the gelation period G' increases sharply due to the formation of elastic effective intermolecular cross-links which give rise to a growing network. In the postgelation period the G'-curve levels off, indicating the end of the gelation process.

Type b. These were creep experiments carried out to optimize the applied stress used in the frequency-oscillation experiments (see type c) and to check for slip effects. On the one hand, the lower limit of the applied stress is determined by the sensitivity of the instrument. On the other hand, the upper limit of the applied stress will be influenced by the linear viscoelastic range and the presence of slip effects. Due to the presence of a thin solvent layer at the surface of some dex-gma hydrogels (only for the densest hydrogels), it was necessary to check if there were any slip effects of the measuring plates. Applying a low stress may prevent slip effects. Figure 3 illustrates the results of creep experiments. Curve A is the result of a creep experiment performed on a loosely cross-linked dex-gma hydrogel applying a stress of 80 Pa. Soon after starting the experiment the deformation shows its equilibrium value. After removing the stress, the deformation recovers completely. This behavior is typical for elastic materials if no slip effects are present during the measurement. Curve B shows the result of a creep experiment performed on a strongly

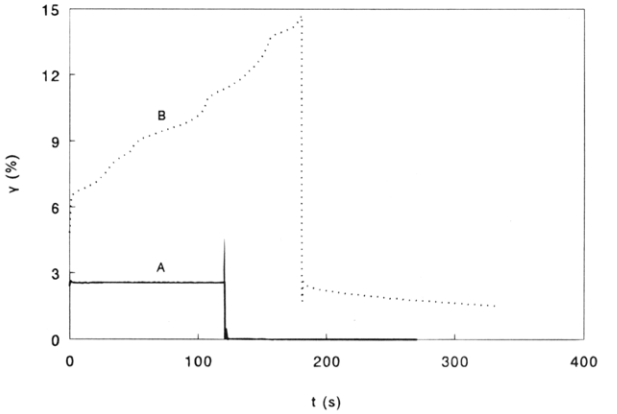

Figure 3. Deformation profile during a creep and a relaxation test on a dex-gma hydrogel in the absence (A) and the presence (B) of slip effects.

cross-linked dex-gma hydrogel applying a stress of 100 Pa: the deformation does not reach an equilibrium value due to the presence of slip effects which manifest themselves as an irreversible viscous flow. It is important to emphasize that the absence of slip effects in creep experiments is not an absolute proof for the absence of slip effects in frequency-oscillation experiments performed at the same stress as the creep experiment, because the continual movement of the upper plate during a frequency-oscillation experiment increases the chances of slip effects.

Type c. The G_e-values of the dex-gma hydrogels were obtained from frequency-oscillation experiments performed on hydrogels in the postgelation period applying a stress determined by the procedure explained under type b. At each frequency, G' was measured five times.

Dynamic viscosity measurements were carried out on the dex-gma solutions which were used to prepare the dex-gma hydrogels. The experiments were performed at 20 °C on a controlled-strain Haake CV100-RV100-RC20 rheometer using a self-designed coaxial cylindric Mooney−Ewart system. A standard oil was used to calibrate the instrument.

Analytical Determination of the Sol Fraction. The sol fraction of the dex-gma hydrogels was determined analytically. Cylindrical hydrogels were prepared in tubes. Weighed amounts of relaxed dex-gma hydrogels were immersed in 10 mL of solvent and allowed to swell at 20 °C. To extract the polymer chains not incorporated in the network (i.e., the sol chains), the solvent was renewed twice a week. During the extraction period, the samples were shaken continuously. A gel permeation chromatography instrument (GPC) was used to determine the polymer concentration in the extraction solvent. The chromatographic system consisted of a pump (Waters 510), an injector (Waters UK6), a differential refractometric detector (Waters 410), and a chromatographic column (Ultrahydrogel 250). The polymer concentration was calculated from the area under the dex-gma peak. Four standard lines of the four corresponding dex-gma polymers were used (concentration between 0.06 and 6 mg/mL). In this concentration range we got an excellent reproducibility and a good linear correlation (correlation coefficient for unweighted linear regression > 0.9996) for each standard line.

Swelling Experiments. To characterize the swelling behavior of the dex-gma hydrogels, weight and volume of the hydrogels in the relaxed and in the swollen state were measured. The swelling measurements were performed on the cylindrical dex-gma hydrogels used in the analytical sol fraction experiments.

The volume of the hydrogels was determined using Archimedes' buoyancy principle:[16]

$$V_{g,r} = \frac{W_{a,r} - W_{h,r}}{\varrho_h} \tag{5}$$

$$V_{g,s} = \frac{W_{a,s} - W_{h,s}}{\varrho_h} \tag{6}$$

In eqs 5 and 6 $V_{g,r}$ and $V_{g,s}$ are respectively the volume of the hydrogel in the relaxed and the swollen state, while $W_{a,r}$ and $W_{h,r}$ are respectively the weight of the hydrogel in air and in hexane (being a nonsolvent for the dex-gma hydrogels). ϱ_h is the density of hexane at the experimental temperature (0.659 g/mL at 20 °C).

The polymer volume fractions of the dex-gma hydrogels in both the relaxed ($\nu_{2,r}$) and the swollen state ($\nu_{2,s}$) were calculated using the following relationships:

$$\nu_{2,r} = V_p/V_{g,r} \tag{7}$$

$$\nu_{2,s} = V_p/V_{g,s} \tag{8}$$

In eqs 7 and 8, V_p represents the volume of the dry polymer.[16] V_p was calculated by multiplying the weight of the dry dex-gma polymer (present in a hydrogel) by the specific volume of dex-gma (see below). As extraction of the sol fraction takes place during the swelling experiments, and as the removal of material affects the value of $\nu_{2,s}$, to calculate $\nu_{2,s}$, we corrected the dex-gma weight by substracting the extracted polymer mass (as measured by GPC experiments) from the initial dry dex-gma weight.

Determination of the Specific Volume of Dex-gma. The specific volume ($\overline{\nu_2}$) of dex-gma was determined using the following relation:[17]

$$\overline{\nu_2} = \frac{1}{\dfrac{m_2}{m_1 + m_2}}\left[\frac{\varrho_1 - \dfrac{m_1}{m_1 + m_2}\varrho_{12}}{\varrho_1\varrho_{12}}\right] \tag{9}$$

In eq 9 m_1 and m_2 are respectively the solvent mass and the (dry) polymer mass of the dex-gma solutions, while ϱ_1 and ϱ_{12} are respectively the density of the solvent and the dex-gma solution. To determine $\overline{\nu_2}$, the density of strongly diluted dex-gma solutions was measured as a function of the dex-gma concentration. The density measurements were performed at 20 °C using a DMA 601 instrument (Anton Paar) calibrated with air and degassed water (accuracy ± 0.000004 mg/mL). We calculated $\overline{\nu_2}$ for dex-gma with DS $^1/_{10}$ and found it to be 0.74 ± 0.02 mL/g.

Results and Discussion

For each dex-gma hydrogel G_e was measured on three samples prepared independently. Typically, G' was independent of the applied frequency (G' equaled G_e), indicating the existence of a real rubbery network (see Figure 4). Figure 5 represents the measured G_e-values as a function of the polymer concentration in the relaxed dex-gma hydrogels. Roughly speaking, as expected, G_e increases as the dex-gma concentration and the degree of gma substitution increases due to a higher concentration of elastic effective network chains (see eq 2).

Although the eqs 2–4 are essentially valid for ideal as well as for nonideal networks, to calculate structural parameters (like M_c) from G_e, it is necessary to analyse ν, taking into account the nonidealities of the concerned network. In the 1940s, Flory had already presented a statistical theory concerning the cross-linking of primary polymer chains into a network having nonideal properties due to the presence of dangling ends and the presence of a sol fraction. Flory assumed tetrafunctional junctions and monodisperse polymer chains.[8] Only recently, te Nijenhuis made an extension of this theory, taking into account the polyfunctionality of the junctions and the polydisperse polymer chains.[12–15] He showed that the following expression is valid for an

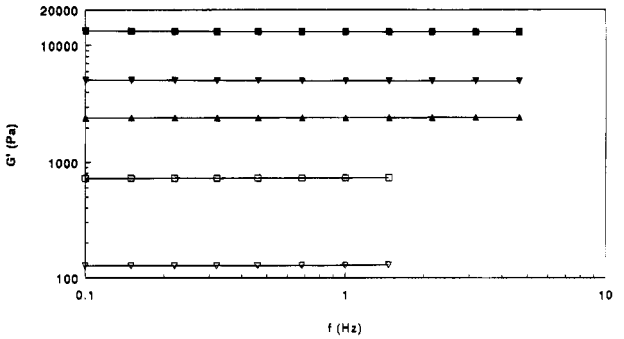

Figure 4. Shear storage modulus (G') as a function of the applied frequency (f) for dex-gma hydrogels (DS $^1/_{10}$) with different concentrations (mg/mL): 64.2 (▽), 79.7 (□), 98.3 (▲), 117.3 (▼), 145.7 (■). The standard deviation is not indicated as the error bar is smaller than the marker size.

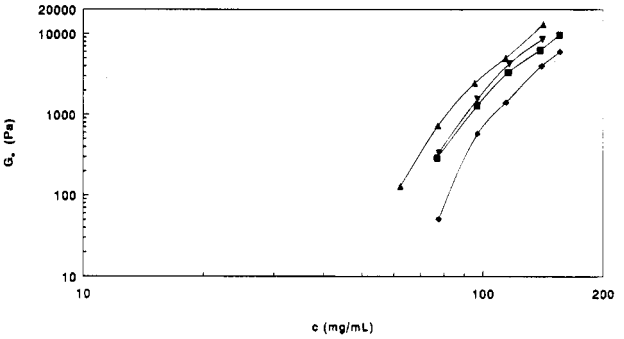

Figure 5. Equilibrium shear modulus (G_e) of the hydrogels as a function of the concentration (c) and the degree of gma substitution (DS): $^1/_{10}$ (▲), $^1/_{22}$ (▼), $^1/_{23}$ (■), $<^1/_{23}$ (◆). The standard deviation is not indicated as the error bar is smaller than the marker size.

affine network:

$$G_e = 2\frac{cRT}{M_n''}(1 - w_s)\left[\left(\frac{M_n''}{M_c''}\right)\frac{f-2}{f} - 1\right] \tag{10}$$

In eq 10 M_n'' is the number-average molecular weight of the primary polymer (being the dex-gma chains) *present in the network fraction*, while M_c'' is the average molecular weight between cross-links *in the network*. Considering eq 10, it is clear that, even if f and the molecular weight of the (original) primary polymer chains (M_n) are known, the measurement of G_e is not sufficient to calculate M_c'' as there are still two unknown parameters: w_s and M_n''. On the basis of statistical considerations, te Nijenhuis derived the equation which relates w_s, M_n'', and M_c'', taking into account the molecular weight distribution of the primary polymer and the functionality of the junctions.[12–15] Consequently, it became possible to write eq 10 only as a function of w_s:

$$G_e = 2\frac{cRT}{M_n}(1 - w_s^{0.5})\left(\frac{1 - w_s^{0.5f}}{1 - w_s^{0.5f-1}}w_s^{-0.5}\frac{f-2}{f} - 1\right) \tag{11}$$

Equation 11 is only valid for affine networks. To account for a nonaffinelike behavior, by using eqs 1 and

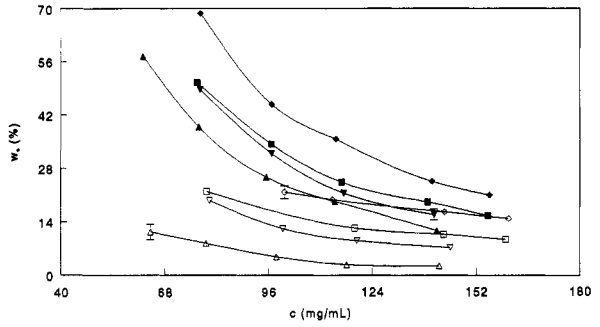

Figure 6. Respectively the sol fraction calculated from G_e (w_s-(G); $f = 4$; closed symbols) and the sol fraction determined analytically (w_s(ANAL); open symbols) of the hydrogels as a function of the concentration (c) and the degree of gma substitution (DS): $^1/_{10}$ (▲,△), $^1/_{22}$ (▼,▽), $^1/_{23}$ (■,□), $<^1/_{23}$ (◆,◇). The standard deviation is not indicated when the error bar is smaller than the marker size.

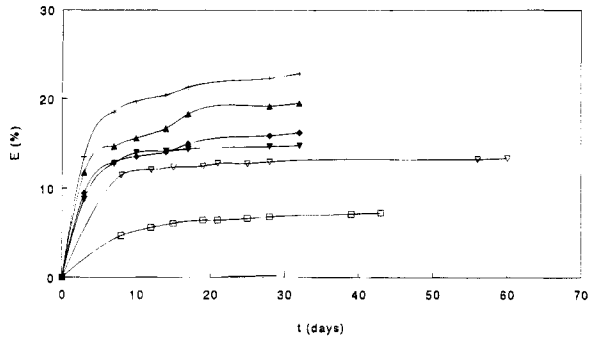

Figure 7. Extracted fraction (E) as a function of the extraction time (t). The concentration (mg/mL) and the DS of the hydrogels are respectively as follows: 100.3, $<^1/_{23}$ (+); 113.2, $<^1/_{23}$ (▲); 143.5, $<^1/_{23}$ (◆); 160.8, $<^1/_{23}$ (▼); 119.3, $^1/_{23}$ (▽); 160.0, $^1/_{23}$ (□).

4, eq 11 can be written as:

$$G_e = \left(1 - h\frac{2}{f}\right) \times 2\frac{cRT}{M_n}(1 - w_s^{0.5})\left(\frac{1 - w_s^{0.5f}}{1 - w_s^{0.5f-1}} w_s^{-0.5}\frac{f-2}{f} - 1\right) \quad (12)$$

By means of eq 12, the w_s-values were calculated from G_e (w_s G)). Figure 6 represents the results. Tetrafunctional junctions ($f = 4$) and an affinelike behavior ($h = 0$) were assumed. It is clear that the lowest w_s(G)-values, or higher "network fractions", were found for the most concentrated dex-gma hydrogels with a high degree of gma substitution. To check the validity of the w_s(G)-values, the sol fraction of all the dex-gma hydrogels was determined analytically as described in the Experimental Section. To ascertain the end of the sol extraction, analytical measurements were carried out on the extraction solvent as a function of the extraction time (see Figure 7). The results of the analytical work are also summarized in Figure 6. The w_s(ANAL)-values show the same trend as the w_s(G)-values: lower w_s-(ANAL)-values were found for dex-gma hydrogels with a higher dex-gma concentration and with a higher DS. However, the w_s(ANAL)-values are systematically lower than the w_s(G)-values. Figure 8 represents the ratio w_s-(ANAL)/w_s(G) for all the hydrogels. The difference between w_s(ANAL) and w_s(G) increases as the dex-gma concentration of the hydrogels decreases and as the degree of gma substitution increases.

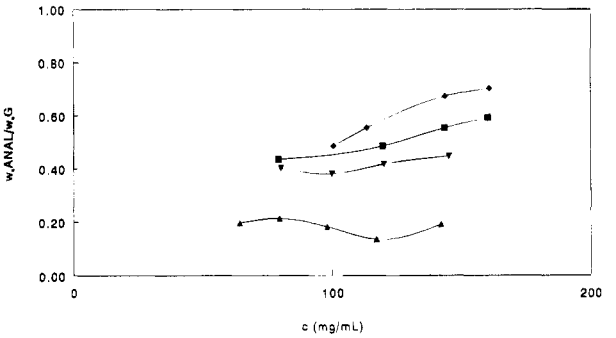

Figure 8. Ratio w_s(ANAL)/w_s(G) of the hydrogels as a function of the concentration (c) and the degree of gma substitution (DS): $^1/_{10}$ (▲), $^1/_{22}$ (▼), $^1/_{23}$ (■), $<^1/_{23}$ (◆).

For the interpretation of Figures 6 and 8 it is important to stress that a complete extraction of the sol fraction was assumed. Possibly, some of the soluble components were not able to diffuse out of the swollen network which is difficult to check experimentally. However, as the reproducibility of the w_s(ANAL)-values is quite good, it seems that incomplete sol extraction was not a real problem. If present, a worse reproducibility would be expected. Moreover, the sol fraction did not increase after cutting the hydrogels to small pieces.

As the w_s(ANAL) values differ from the w_s(G)-values, it is clear that the dex-gma networks do not completely resemble the structure of the network in the theoretical model. To find out the reason for the differences between w_s(G) and w_s(ANAL), we evaluated the assumptions of the theoretical model which seemed rather obscure in the case of the dex-gma hydrogels.

First, for the comparison between w_s(G) and w_s-(ANAL) (Figures 6 and 8), tetrafunctional junctions were assumed for calculating w_s(G). On the one hand, due to the radical cross-linking of the dex-gma chains, junctions with a functionality higher than 4 could be expected. On the other hand, due to the rather long structure of the "cross-linking chains" (i.e., 2 linked gma groups consisting of 16 skeletal bonds) which may act as elastically-effective chains, the dex-gma networks may be considered as trifunctional bimodal networks.[18] Moreover, due to α-1,2 and α-1,3 bonds present in the dextran chains, which cause the branched structure of the chains, there might be some regions in the dex-gma networks which act as trifunctional junctions. Recalculation of w_s(G) using higher functionalities ($f > 4$) gives us even larger differences with the w_s(ANAL)-values, while considering trifunctional junctions decreases the difference between the w_s(G)- and w_s(ANAL)-values. Roughly speaking, considering junctions with other functionalities may also contribute to the differences between w_s(G) and w_s(ANAL) but cannot completely explain it.

Second, for the w_s(G)-calculation an affinelike behavior of the dex-gma hydrogels was assumed. Experimentally, it is rather difficult to check the validity of this assumption.[19] As already mentioned in the Introduction, it can be expected that real networks probably do not show an affinelike behavior but a deformation behavior somewhere between the deformation properties of affine and phantom models. On the basis of eq 12, we checked the possibility if w_s(G)-values, equal to the w_s(ANAL)-values, could be calculated by using h-values between zero and 1. Meaningful h-values ($0 \leq h \leq 1$) were only found in the case of the strongly concentrated hydrogels (with low DS). These considerations indicate that a nonaffinelike behavior of the

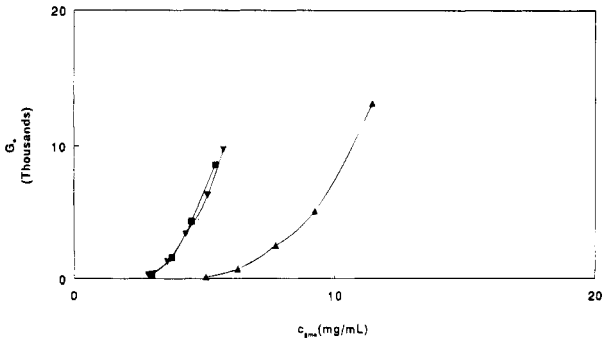

Figure 9. Equilibrium shear modulus (G_e) of the hydrogels as a function of the gma concentration (c_{gma}) and the degree of gma substitution (DS): $^1/_{10}$ (▲), $^1/_{22}$ (▼), $^1/_{23}$ (■).

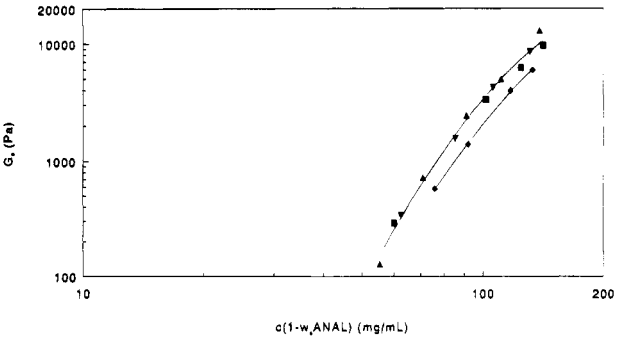

Figure 10. Equilibrium shear modulus (G_e) of the hydrogels as a function of the network concentration ($c(1-w_s(ANAL))$) and the degree of gma substitution (DS): $^1/_{10}$ (▲), $^1/_{22}$ (▼), $^1/_{23}$ (■), $<^1/_{23}$ (◆).

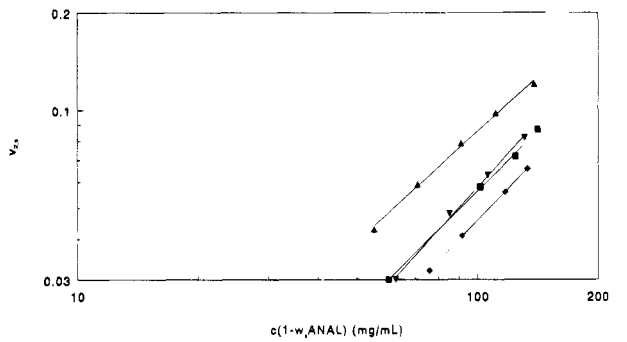

Figure 11. Polymer volume fraction in the swollen state of the hydrogels ($v_{2,s}$) as a function of the network concentration ($c(1-w_s(ANAL))$) and the degree of gma substitution (DS): $^1/_{10}$ (▲), $^1/_{22}$ (▼), $^1/_{23}$ (■), $<^1/_{23}$ (◆). The standard deviation is not indicated as the error bar is smaller than the marker size.

dex-gma hydrogels may also contribute to the deviations observed between w_s(G) and w_s(ANAL).

Third, in his theoretical model te Nijenhuis assumes that entanglements do not contribute to G_e. From viscosity (η) measurements performed on the dex-gma solutions (used in the preparation of the dex-gma hydrogels) at different shear rates (from 1 up to 100 s^{-1}) all the solutions showed a low viscosity (<20 mPa·s) and a Newtonian behavior. Moreover, there was a linear correlation between log η and log c (slope $\approx$ 1). Although these features are not a proof for the absence of entanglements in the dex-gma solutions, they do indicate the existence of rather diluted polymer solutions without strong intermolecular interactions. This satisfies the assumption of te Nijenhuis.

Fourth, te Nijenhuis also assumed that intramolecular cross-links are absent. For the dex-gma hydrogels this is highly unlikely. Figure 1 illustrates intra- and intermolecular cross-links. As the network chains between intramolecular cross-links create no elasticity, this feature could be used to interpret Figure 8: a dex-gma hydrogel with a given w_s (=w_s(ANAL))-value will show a lower G_e-value (i.e., a higher w_s(G)) if not only intermolecular cross-links are present but also intramolecular cross-links. Although it is possible to calculate reasonably the presence of certain network defects like the sol fraction and dangling ends, it is extremely difficult to know the amount of intramolecular cross-links. However, Tonelli and Helfand presented a rather theoretical work concerning the presence of intramolecular cross-links in polyisoprene networks.[20,21] The study showed that the probability of intramolecular cross-linking is not negligible.[22,23] Actually, loops are expected when the networks are prepared by cross-linking diluted, highly substituted, low molecular weight polymer solutions. The lower the polymer concentration and the higher the degree of gma substitution, the higher the probability for intramolecular cross-linking. These features are in agreement with the results presented in Figure 8: the lower the dex-gma concentration of the hydrogels and the higher the degree of gma substitution, the larger the difference between w_s(G) and w_s(ANAL) (or the larger the difference between the measured G_e-value and the expected G_e-value based on the network model). An additional argument for intramolecular cross-linking is the relation between G_e and the gma concentration (i.e., the concentration of the cross-linking agent). Although accurate measurements to determine the minimal gma concentration necessary for gelation were not performed, Figure 9 indicates that a higher minimal gma concentration is necessary when the DS of the hydrogels increases. Moreover, comparing dex-gma hydrogels with the same gma concentration, higher G_e-values (i.e., the concentration of the intermolecular cross-links is higher) were measured for hydrogels prepared by cross-linking dex-gma with a lower DS. As it was impossible to determine the DS of the lowest substituted dex-gma (see the Experimental Section), Figure 9 only represents the results of three dex-gma batches.

Figure 10 represents G_e as a function of the network concentration of the dex-gma hydrogels. The network concentration is the total dex-gma concentration in the hydrogel multiplied by (1 − w_s(ANAL)). As opposed to Figure 5, the G_e values of the hydrogels with DS $^1/_{10}$, $^1/_{22}$, and $^1/_{23}$ lie on the same curve, whereas the G_e-values of the hydrogels with DS < $^1/_{23}$ are somewhat smaller. The phenomenom presented in Figure 10 may be explained as follows: although a DS increase increases the network fraction in the hydrogels (i.e., decreases the sol fraction; see Figure 6), above a certain level it does not further increase the number of intermolecular cross-links per unit of mass present in the network fraction. This can be understood if one accepts that above a certain degree of gma substitution intramolecular cross-linking strongly dominates in comparison with intermolecular cross-links.

In Figure 11 the polymer volume fraction of the swollen hydrogels ($v_{2,s}$) is represented. As opposed to the elastic properties (Figure 10), dex-gma hydrogels with the same network concentration but with a different DS have another swelling behavior. In order to elucidate this swelling behavior, the Flory polymer–solvent interaction parameter (χ) was calculated using the equation represented by Queslel and Mark valid for

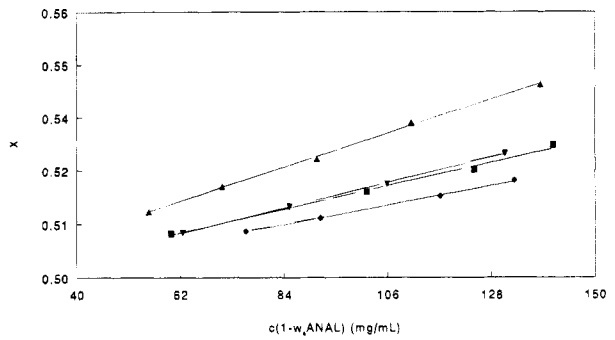

Figure 12. Polymer–solvent interaction parameter of the hydrogels (χ) as a function of the network concentration ($c(1-w_s(\mathrm{ANAL}))$) and the degree of gma substitution (DS): $^1/_{10}$ (▲), $^1/_{22}$ (▼), $^1/_{23}$ (■), $<^1/_{23}$ (◆).

affine networks:[9]

$$\ln(1-\nu_{2,s})+\nu_{2,s}+\chi\nu_{2,s}^{\,2}=-\frac{G_e}{RT}V_1\left(1-\frac{2}{f}\right)^2\left[\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3}-\frac{2}{f}\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)\right] \quad (13)$$

In eq 13 V_1 is the molar volume of the swelling agent. The results of the χ-calculation are represented in Figure 12 assuming tetrafunctional junctions. It is clear that the degree of gma substitution has an influence on the calculated χ-value. As the chemical nature of the gma molecules is different from the nature of the dextran chain, it is reasonable to believe that, in an aqueous solvent, increasing the gma content increases the χ-value. Also the appearance of the hydrogels agrees with their χ-profile: although no turbidimetric measurements were carried out, the most concentrated dex-gma hydrogels, especially those prepared with the strongly substituted dex-gma, seemed to show a low turbidity, indicating the presence of heterogeneities which may have important consequences on the structure of the hydrogels. In comparison with the reported χ-value for dextran (0.474 for dextran, with an average molecular weight of 9000, in water at 37 °C), the calculated χ-values seem to be acceptable.[24]

Finally, due to the nonideal network structure of the dex-gma hydrogels, it is extremely difficult to characterize the dex-gma network structures in all detail. Although a main goal of this work was to calculate precisely the average molecular weight between the junctions by using a theoretical model, it seems from the preceding explanation that it is difficult and rather risky. Besides rheological, swelling, and analytical sol fraction experiments, further experimental work is necessary on the functionality of the junctions, the affine versus phantom behavior, the influence of the concentration, and the DS on the χ-parameter. Of especially great value would be the use of theoretical models which would allow the influence of intramolecular cross-links on the elastic and the swelling properties of the hydrogels to be evaluated. Theoretical work to optimize the model of te Nijenhuis started recently. The structural network properties of dex-gma hydrogels discussed in this presentation will contribute to understanding the fundamentals of the diffusion of macromolecular probes in the dex-gma hydrogels. It would be valuable to study the influence of both intra- and intermolecular cross-links on the probe diffusion.

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