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THERMAL AND RHEOLOGICAL PROPERTIES OF GELATIN–DEXTRAN HYDROGELS

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Abstract

Hydrogels prepared by crosslinkage of gelatin with dextran dialdehyde have been characterized by dynamic shear oscillation measurements at small strain. Isothermal as well as temperature scan measurements were performed. The results obtained demonstrated that the final polymer network is a result of a chemical gelatin–dextran dialdehyde interaction as well as a gelatin–gelatin (physical association) and a polymer-solvent interaction. This balance is strongly dependent on the composition of the system, the polymer concentration, the storage temperature and the storage time.

We found that a short cryogenic treatment at –20°C of physically structured gels, significantly increases the chemical crosslinkage. DSC measurements at low cooling rate confirm these results and demonstrate a chemical reaction enthalpy contribution.

Keywords: calorimetry, dextran, gelatin, hydrogels, rheology

Introduction

Recently, polymer hydrogels are used intensively as proper materials for biomedical applications including contact lens materials [1], artificial tendons [2], materials for tissue engineering [3], drug delivery systems [4–6] etc. Hydrogels can be obtained from hydrophilic monomers, prepolymers or polymers.

In a previous paper [7] we have described the synthesis of hydrogels prepared by reaction of gelatin with partially oxidized dextran. The crosslinkage is predominantly due to Schiff's base formation between the ε-amino groups of lysine or hydroxylysine side groups of gelatin and dextran dialdehyde:

$$\text{Gel–NH}_2 + \text{Dex–CHO} \leftrightarrow \text{Gel–N{=}CH–Dex} + \text{H}_2\text{O}$$

Using the low shear rotation viscometer it has been demonstrated that the rate of gelation during reaction between gelatin and dextran dialdehyde depends on the molecular weight and degree of oxidation of dextran, the nature of gelatin and the reaction conditions. Gelation could only be followed over a short time period since after onset of gelation the gel structure ruptures during rotation. In continuation of

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this work the rheological and mechanical properties of the hydrogel were examined by dynamic mechanical measurements using a shear oscillating rheometer.

The dynamic mechanical properties of gelatin hydrogels [8–25] and gelatin-polysaccharide systems [26–30] have received limited attention in literature. This work aims to investigate the rheological properties of chemical crosslinked gelatin–dextran hydrogels using oscillation test measurements at small deformation. The temperature dependence of the elastic modulus G' of gelatin–dextranox hydrogels will be discussed.

Experimental

Materials

Gelatin with bloom strength 203, isoelectric point (IEP)=7.0, $[\eta]=2.87$, $pH=5.3$ was supplied by Systèmes Bio Industries, Benelux (Ghent, Belgium). Dextran was obtained from Pharmacia (Uppsala, Sweden), MW=70000. The gelatin was used as received. Dextran was oxidized by controlled amounts of sodium periodate as previously described by Ruys [31].

Hydrogel preparation

Hydrogels were prepared by mixing 10 wt.% buffered aqueous solutions of gelatin and oxidized dextran. The typical weight ratio was 1:1; the mixture was kept for 30 s at 40°C and then stored at a selected temperature in the range from 4 to 40°C for a chosen period of time.

Rheological measurements

The rheological measurements at oscillatory shear deformation on the gelatin hydrogels were carried out with a rheometer Rheolab MC20 (Physica, Germany) using parallel plates of 50 mm diameter and a plate-to-plate distance of about 1 mm.

The temperature dependence of the storage (elastic) modulus was determined by oscillatory shear deformation and temperature scan in the range from 17 to 50°C (heating rate 2.75°C min^{-1}) at constant trequency (f=1 Hz) and constant shear strain ($\gamma=0.05$, 1.88 mrad).

DSC measurements

Calorimetric studies were run with a DSC-7 (Perkin Elmer) at a rate of cooling 1 and 10°C min^{-1} using hermetic closed sample pans.

Results and discussion

Gelatin forms transparent homogeneous hydrogels in a concentration range from 5 to 20 wt.%. The hydrogels composed of gelatin and non oxidized dextran are heterogeneous and opaque at room temperature, due to phase separation of gelatin and dextran which are incompatible [32–33]. On the contrary, the chemical crosslinked hydrogels obtained from gelatin and oxidized dextran are transparent and homogeneous at room temperature.

The hydrogel formation (gelation) of gelatin–dextran dialdehyde in aqueous solution involves basically two different strong interactions. The first one is associated with the chemical crosslinking of gelatin and dextran dialdehydes (gelatin–dextran chemical interaction) and the second is based on the ability of gelatin to form polymer network structures that are stabilized by secondary forces (physical crosslinking; gelatin–gelatin physical interaction).

The influence of different factors (degree of oxidation of dextran, gelatin–dextran ratio, polymer concentration, storage temperature and storage time) on the viscoelastic properties of gelatin hydrogels were studied in more detail. For comparison, the dynamic rheological properties of a gelatin hydrogel and a combination of gelatin with non oxidized dextran [34] were also investigated.

Gelatin hydrogels

It is known that the physical crosslinking (gelation) occurs when a heated, aqueous solution of gelatin is cooled below the sol–gel transition temperature [8].

Rheological measurements indicate that the "melting point" of gelatin hydrogels depends on storage conditions. As is shown in Fig. 1 (curve 1) gels stored at 20°C have a higher melting point than gels obtained at 4°C. One can speculate that the

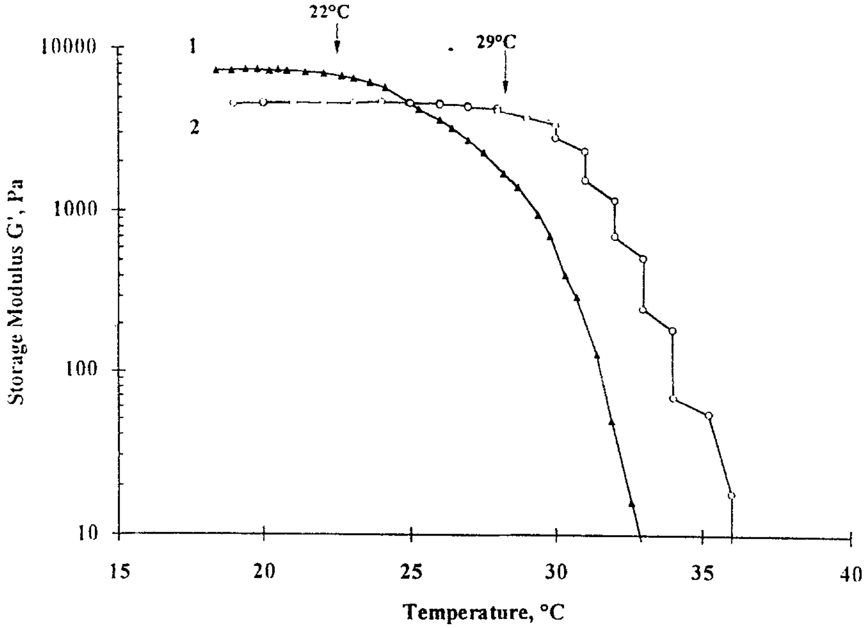

Fig. 1 Temperature dependence of storage modulus G' of gelatin hydrogels; 10 wt.%; $pH=7$; storage time 5 days at 4°C (curve 1) or at 20°C (curve 2). Oscillation shear deformation $\gamma=0.05$; frequency $f=1$ Hz

physical network formed during storage at 20°C is thermodynamically more stable (larger domains). On the other hand, gels formed at 4°C may have a higher number of small size interactions leading to an increased storage modulus G' below gel-sol transition temperature (Fig. 1 curve 2).

Gelatin–dextran dialdehyde hydrogels

Hydrogels prepared from gelatin and dextran dialdehyde are transparent gels with a storage modulus higher than that for the gelatin–dextran gels [34]. This is undoubtedly due to the chemical interaction between gelatin and the dextran dialdehyde which decreases significantly the phase separation.

The results of isothermal rheological measurements at room temperature demonstrate the influence of both, chemical and physical crosslinking of the gelatin–dextran dialdehyde hydrogels. The temperature scan of the hydrogel specimens below and above melting point allows us to identify the contribution of chemical and physical crosslinkage separately.

The gelatin hydrogels that are formed only by physical gelation (gelatin and combination of gelatin with non oxidized dextran) show high G'-values below melting point. However the elastic modulus decreases rapidly to very low values, when the temperature increases above melting point (33–35°C) (Fig. 1). Figure 2 represents the temperature dependency of the storage modulus G' for the gelatin–dextran

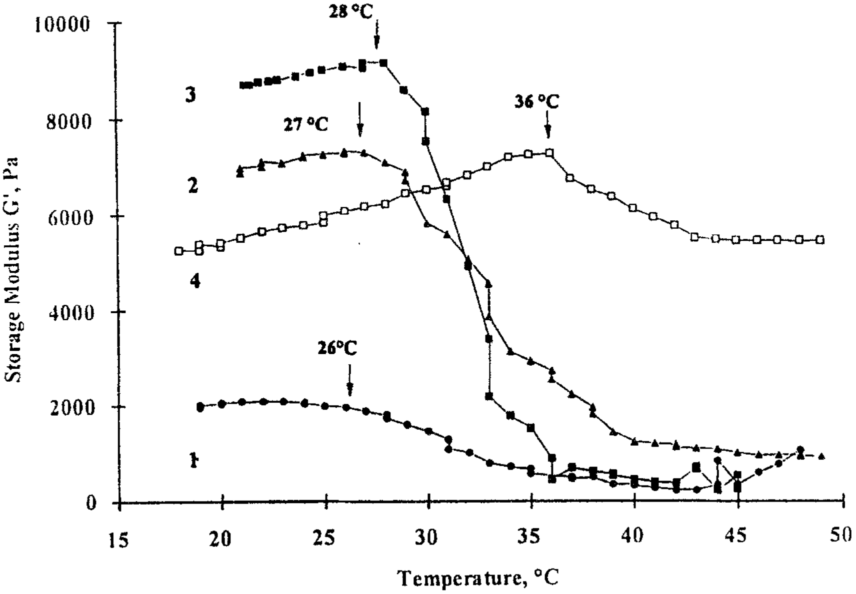

Fig. 2 Temperature dependence of storage modulus G' of gelatin–oxidized dextran hydrogels; 1:1 wt. ratio; polymer concentration 10 wt.% ; oxidation degree of dextran 5% (curve 1), 10% (curve 2), 20% (curves 3 and 4); storage time 10 days at 4°C (curves 1–3) and 20°C (curve 4); Oscillation shear deformation $\gamma=0.05$; frequency $f=1$ Hz

dialdehyde hydrogels with different composition and prepared at 4 or at 20°C. Oxidation degree of dextran changes from 5 to 20%; the gelatin–dextran dialdehyde ratio was 1:1. As can be seen, below melting point the storage modulus increases, as the concentration of dialdehyde groups in dextran increases. The gel–sol transition temperature increases slightly but measurably in the same way (Fig. 2, curves 1–3). The increase of storage temperature from 4 to 20°C leads to a decrease of the *G*′-modulus but the melting temperature is increased significantly. Above the melting point, *G*′ decreases rapidly to low values for hydrogels that are stored at 4°C (Fig. 2, curves 1–3). These results show the importance of the physical bonding in gelatin–dextran hydrogels, which contributes far more to the viscoelastic properties of the hydrogels than the chemical crosslinks alone, if prepared under the given conditions of gelation.

Generally, above melting point the *G*′-modulus of the hydrogels and the contribution of the chemical crosslinks increase with increasing the degree of oxidation of dextran and the storage temperature. For comparison Fig. 2 (curves 3 and 4) shows the temperature dependency of the storage *G*′-modulus of gelatin–dextran dialdehyde hydrogels with the same composition (20% oxidation of dextran), but differing in storage temperature (4 and 20°C) for a same storage time. As can be seen, above the melting point the *G*′ increases significantly at higher storage temperature. It is anticipated that chemical crosslinkage between gelatin and dextran dialdehyde is far more effective at 20°C than at 4°C.

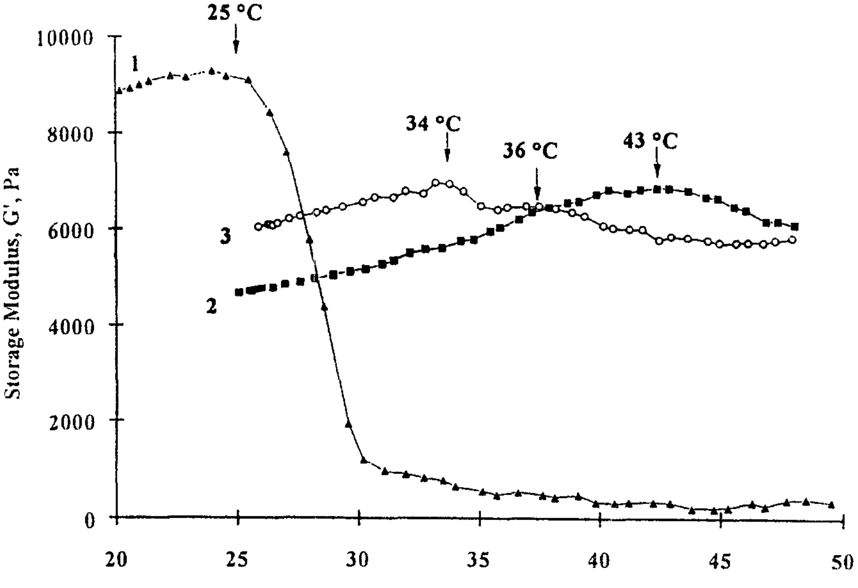

Fig. 3 Temperature dependence of storage modulus *G*′ of gelatin–oxidized dextran hydrogels; 1:1 wt. ratio; polymer concentration 10 wt.%; oxidation degree of dextran 20%; storage time: 10 days at 4°C (curve 1); 7 days at 20°C and 3 days at 4°C (curve 2); 7 days at 4°C and 3 days at 20°C (curve 3); Oscillation shear deformation $\gamma=0.05$; frequency $f=1$ Hz

The above data show that different gel structures and consequently different viscoelastic properties can be obtained depending on the reaction-storage conditions. This is further illustrated in Fig. 3 which represents the temperature dependence of the G' for a number of gels prepared from the same reagents, but after different time–temperature conditions. The gel stored 10 days at 4°C has a high G'-value below melting and a very low G'-value after melting (Fig. 3, curve 1). The gel allowed to react for 7 days at 20°C and subsequently 3 days at 4°C has less but structurally more perfect physical junctions and more chemical structuring (higher G'-value above 30°C) (Fig. 3, curve 2). For the gel that was first stored 7 days at 4°C and then 3 days at 20°C the G'-modulus below melting is higher than in case 2 but still lower than in case 1 (Fig. 3, curve 3). These results indicate that, when stored at 4°C, chemical reaction is suppressed and gel structuring is mainly due to aggregation of the gelatin chains. When first stored at 20°C and then at 4°C, the contribution of physical structuring appears to be less pronounced due to increased rate of chemical immobilization of the gelatine chains. Curves 2 and 3 show a comparable G'-value at 50°C. The two melting points in curve 3 are probably due to two different types of association of gelatin formed under different storage conditions.

In all above experiments the reagents were quickly mixed, shortly kept at 40°C and then stored either at 4 or at 20°C. It can be anticipated that the increase of the

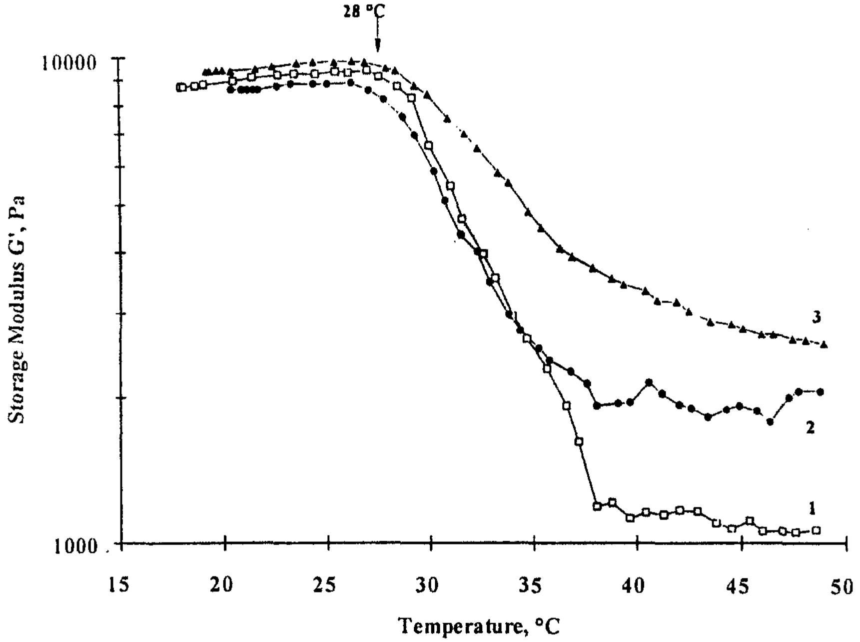

Fig. 4 Temperature dependence of storage modulus G' of gelatin–oxidized dextran hydrogels; 1:1 wt. ratio; polymer concentration 10 wt.%; oxidation degree of dextran 20%; storage time: 2 min at 40°C and 9 days at 4°C (curve 1); 15 min at 40°C and 5 days at 4°C (curve 2); 30 min at 40°C and 5 days at 4°C (curve 3). Oscillation shear deformation $\gamma=0.05$; frequency $f=1$ Hz

initial reaction time at 40°C will stimulate chemical reaction under conditions where physical ordering is not possible.

Figure 4 (curves 1–3) clearly indicates that increased reaction time at 40°C results in more chemical crosslinkage and higher G'-values after melting of the physically structured gelatin fraction in the gels.

An alternative method to increase the fraction of chemically crosslinked gel is cryogenic treatment. It was found that low temperature treatment (at –20°C) of physically structured gels (prepared at 4°C) has a dramatic effect on the G'-value above the gel melting point (Fig. 5, curve 2). This unexpected phenomenon can be explained as follows. During cooling at –20°C the reaction system phase separates and water is crystallizing. Hence, the reagent concentration in the polymer phase increases, resulting in a higher rate of crosslinkage as a result of Schiff's base formation. Moreover, the exothermic effect ΔH_c which is illustrated on the DSC curves in Fig. 6, and the local pressure increase, caused by water crystallization, additionally favour the Schiff's base formation. The positive values of the difference between the enthalpy of crystallization (ΔH_c) at first and second cooling of gelatin–dextran dialdehyde hydrogels can be attributed to the enthalpy contribution of the chemical reaction. Additionally, this difference in ΔH_c values increases with increased oxidation degree of dextran (Table 1).

This possibility to enhance chemical crosslinkage by cryogenic treatment is now further explored. More detailed results will be discussed in a forthcoming paper.

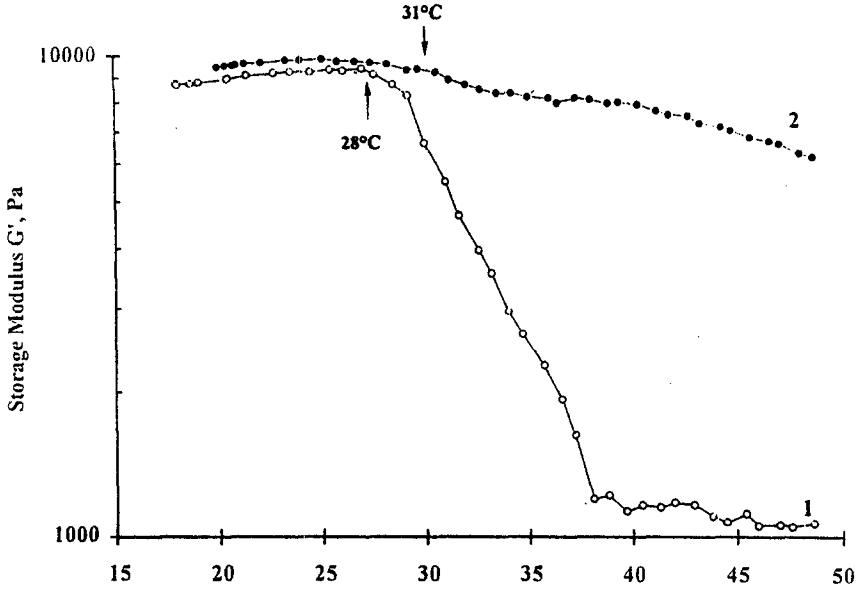

Fig. 5 Temperature dependence of storage modulus G' of gelatin–oxidized dextran hydrogels; 1:1 wt. ratio; polymer concentration 10 wt.%; oxidation degree of dextran 20%; storage time: 9 days at 4°C (curve 1); 8 days at 4°C, 1 day at –20°C and 1 day at 4°C (curve 2). Oscillatory shear deformation $\gamma=0.05$; frequency $f=1$ Hz

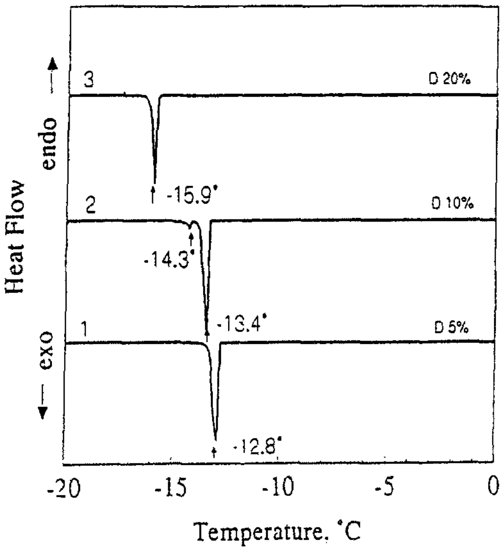

Fig. 6 DSC curves at cooling (1°C min^{-1}) of gelatin–oxidized dextran hydrogels, 1:1 wt. ratio; polymer concentration 10 wt.%; degree of oxidation of dextran: 5% (curve 1); 10% (curve 2); 20% (curve 3); storage time: 13 days at 4°C

Table 1 Enthalpy of crystallization of gelatin and gelatin–dextran dialdehyde hydrogels; storage conditions 15 days at 4°C; cooling rate 10°C min^{-1} from 0° to –20°C

Reagents	Composition	1st cooling ΔH_{c_1}	2nd cooling ΔH_{c_2}	$\Delta H_c = \Delta H_{c_1}-\Delta H_{c_2}$
			J g^{-1}	
Gelatin	10 wt%	248.1	248.8	–0.7
Gelatin+Dextran dialdehyde 10% DO	ratio 1:1–10 wt%	247.7	245.1	2.6
Gelatin+Dextran dialdehyde 20% DO	ratio 1:1–10 wt%	232.8	226.9	5.8
Gelatin+Dextran dialdehyde 60% DO	ratio 2:1–15 wt%	198.8	185.6	13.2

Conclusions

This study has demonstrated that the final polymer network is a result of chemical polymer–polymer interactions (gelatin–dextran dialdehydes) as well as gelatin–gelatin (physical association) and polymer–solvent interactions (gelatin–water and dextran–water). This balance is strongly dependent on the composition of the system, the polymer concentration, storage temperature and storage time.

The contribution of chemical crosslinking on viscoelastic properties of gelatin–dextran hydrogels increases with increasing the storage temperature from 4 to

40°C or after short cryogenic treatment of the hydrogels at –20°C. The contribution of physical association is increased when the storage temperature is decreased and/or the storage time is increased.

The temperature scan measurements of the hydrogels, below and above melting point, allow to identify the contribution of chemical and physical bonding in the final gelatin–dextran network.

This study has clearly demonstrated that the gel structure can be controlled by appropriate selection of the reaction conditions.

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