Physically and Chemically Crosslinked Gelatin Gels

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Summary: In this paper, we examine the rheological and the structural properties of different types of gelatin networks, physical, chemical and both. The physical gel is due to the formation of collagen type triple helices when cooling the solutions. Chemically crosslinked gels are obtained with a reagent, in our case the bis(vinylsulfonyl)methane (BVSM), kindly provided by Kodak Industrie (France). Each BVSM molecule provides two covalent bonds. The chemical reaction was followed by microcalorimetry (MicroDSC III from Setaram, Caluire, France). The relation between shear moduli and crosslinking for the three types of gels is discussed, in relation with theoretical models of randomly crosslinked systems.

Keywords: crosslinking; gelatin; hydrogel; microcalorimetry; physical gel; rheology

Introduction

Gelatin gels are widely used in many applications including food, photographic, pharmaceutical, biomedical and technical applications. Using the same macromolecule in solution (collagen denatured during extraction and dissolved in water) three types of gels can be obtained: the ‘‘physical gel’’ which is thermo-reversible and which is obtained when the solutions are cooled, a ‘‘chemical gel’’ which results from the cross-linking of the chains induced by reagents, which were added to the aqueous solution and hydrogels where both crosslinks are present. In general, the chemical reaction proceeds in the temperature range where the physical gel is not observed, in the high temperature range. The physical gelation is due to the conformational transition of gelatin chains from coil to triple helices. Thus, the two gelation mechanisms can be observed independently. For food and pharmaceutical applications, the thermo-reversible gel is used, the gel is supposed to ‘‘melt’’ in the mouth or in the stomach. For some other applications, like hydrogels both types of crosslinks are necessary. In an intermediate range of temperatures, the two mechanisms of gelation are in competition. In this paper, we examine the rheological and the structural properties of different types of gelatin networks, physical, chemical and both.

Materials and Methods

The gelatin sample used in this investigation is from demineralised ossein, a photographic grade, kindly provided by Rousselot SAS, with $M_w = 165\,000$ g/mole and an index of polydispersity of 2. It was dissolved in a citrate-phosphate buffer. The reagent for chemical crosslinking is the bisvinylesulfonemethyle (BVSM) provided by Kodak Industrie (Chalon-sur-Saône, France). The crosslinker creates covalent C-N links with the amine groups of the gelatin coils (Lysine, Hydroxylysine and Histidine). The reaction is represented schematically on Figure 1.

The methods used were polarimetry, microcalorimetry and rheology. The polarimeter is a Perkin Elmer 341, working at a wavelength of 436 nm, with an optical path of 0.1 dm, the temperature is controlled by an external bath circulating in a jacketed cell. Optical rotation was continuously recorded versus time and temperature. Microcalorimetry was used to follow the

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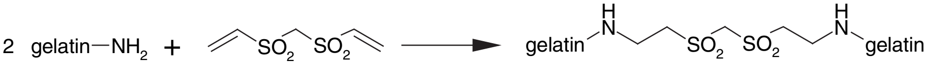

Figure 1.
The BVSM molecule and the crosslinking reaction with gelatin.

chemical reaction (microDSC III from Setaram, Caluire, France). Mixing vessels were necessary, which are made of two chambers: the upper chamber has a volume of 0.2 cm^3 and is filled with the reactant solution and the lower chamber has a volume of 0.55 cm^3 and contains the gelatin solution. A piston separates the two chambers and allows the mixing of the solutions, which initiates the chemical reaction. The crosslinking was studied in the isothermal mode at 40 °C and it is exothermal. Each BVSM molecule possesses 2 double bonds. The enthalpy for formation of one link between gelatin and BVSM was derived from different measurements, where the limiting parameter was the BVSM concentration.

We found:

$$\Delta H_{\text{chem link}} = -40.3 \pm 0.5 \text{ kJ/mole}$$

Rheology was performed with a stress controlled rheometer AR 1000 from TA Instruments. The geometry is a cone and plate with a diameter of 6 cm and an angle of 2°. A lid with a solvent trap is used to avoid evaporation. The temperature was controlled by a Peltier device with an accuracy of 0.1 °C. The rheometer worked with a controlled strain of 0.5% to be the viscoelastic linear regime in the course of gelation.

Most of the rheological experiments were performed at a frequency of 1 Hz; the shear moduli become independent of frequency after the gel point.

Results and Discussion

This section is divided in three parts:

1. The physical gels
2. The chemical gels
3. The hydrogels, which are both physical and chemical

By controlling temperature and presence of the crosslinker, one can prepare any of these three types. By only cooling gelatin solutions, below room temperature, one makes a physical network, which is reversible in temperature; the crosslinks are triple helices of the collagen type. By keeping the gelatin solution at 40 °C in presence of the reagent, a chemically crosslinked gel is created; the gelatin chains are in the coil conformation. By cooling gelatin solutions containing the reagent, one induces both types of crosslinks; a competition arises then between the physical crosslinking and the chemical crosslinking.

1. The Physical Gels

The investigations of the physical gelation of gelatins of various origins, showed that the shear moduli of the physical gels follow a unique curve, the master curve, where the only parameter which determines the elasticity of the network is the concentration of helices[1,2]. The master curve is independent of molecular weight of gelatin, of temperature, of time and it was established for gelatins extracted from mammalians (skins or bones) and fishes (skins). The amino-acid compositions of these proteins vary with their origin and the extractions can be either basic or acidic, thus the master curve is also independent of these parameters. The master curve illustrates the percolation transition, close to the gel point and far from the gel point, reveals a network made of rigid triple helices connected by flexible links.

2. The Chemical Gels

The chemical gelation was followed by microcalorimetry. When the heat flux released is integrated versus time, the total heat of the reaction is determined. In Figure 2 is plotted the total heat exchanged during the crosslinking reactions, at a fixed gelatin concentration (12% g/cm^3) and by adding various amounts of BVSM: 0.075 %, 0.15 % and 0.3 % g/cm^3. The time scales of the experiments are between 4000 and 7000 s (1 to 2 hours). The amount of links formed can be derived from these experiments using the value of ΔH given before.

The rheological experiments in Figure 3 show the time dependence of the shear and loss moduli at fixed temperature.

The gelation time corresponds to $G' = G''$ for our definition. Gelation times are shorter for large gelatin concentrations. The final storage modulus G' also increases with the concentration of BVSM. Comparison between rheology and microcalorimetry determines the gel point and the shear moduli versus the number of links per gelatin chain, for each gelatin concentration. This is shown in Figure 4. The gelatin concentration has a great influence on the location of the gel point. When the crosslinking starts, the individual links form at first, but the gel point is reached only when the second link is made, with a different chain. Therefore, entanglements between chains play an important role. The sol-gel transition shows a critical exponent, close to the vulcanization predictions.

3. The Hydrogels

Gelatin gels with both chemical and physical crosslinks are "hydrogels". They keep a permanent network, even when helices melt. The important feature, which is generally accepted, is that the method of preparation modifies the final mechanical properties of the gels.

There are three different ways of preparing the hydrogels: i) Protocol 1: first by chemical crosslinking, at high temperature, for a long time and then by cooling the solutions to build the physical gel ii) Protocol 2: the solutions are rapidly quenched at 5 °C, then reheated around room temperature (25 °C) to allow the chemical reaction to take place (first physical, then chemical gelation) iii) Protocol 3: both crosslinks form simultaneously at intermediate temperatures. In order to summarize the overall behavior, we plotted the storage modulus versus helix concentration, in all cases investigated, including the case where there is no crosslinker. The results are

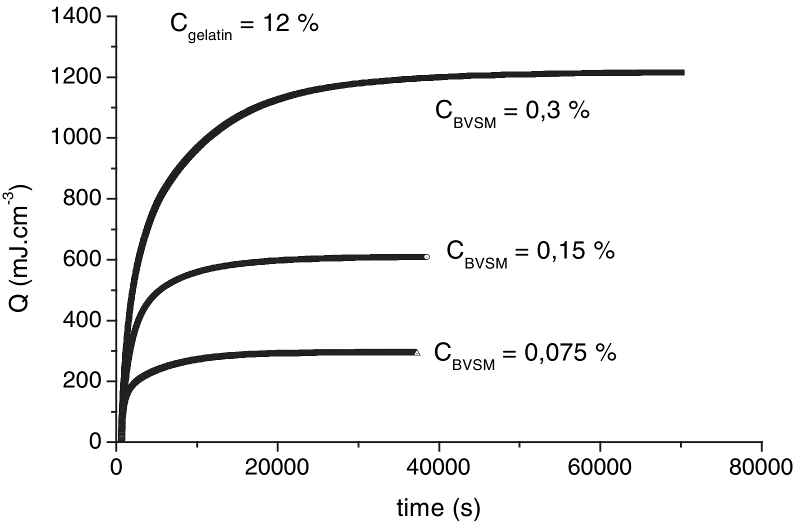

Figure 2.
The total amount of heat released during the crosslinking reaction for a fixed gelatin concentration of 12% and various amounts of BVSM.

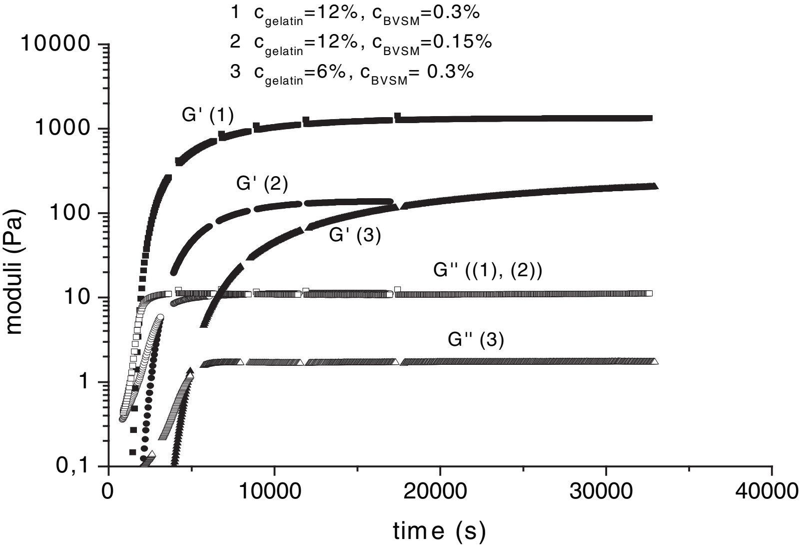

Figure 3.
The time dependence of the shear moduli measured at 1 Hz for two gelatin concentrations, 6% and 12% and two BVSM concentrations, 0.3% and 0.15%. T = 40 °C.

shown in Figure 5. This plot contains data obtained during cooling, or during annealing at constant temperature and during melting. The moduli are dominated by the helix contribution, as it can be seen from the master curve reported also on the plot. There is an extra contribution from the crosslinker, which allows making a stronger gel. Protocol 1, shows precisely the contribution of the chemical gel, randomly crosslinked. For this amount of BVSM, the storage modulus achieved at the end of the reaction, is only 230 Pa. When the helices are formed, the total elasticity at the end of

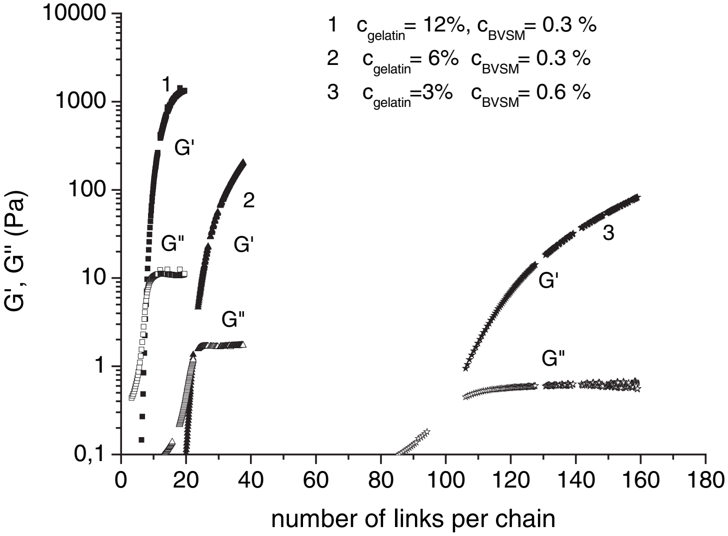

Figure 4.
Moduli versus number of links per gelatin chain ($c_{BVSM} = 0.3\%$ for 12% and 6% gelatin, $c_{BVSM} = 0.6\%$ for 3% gelatin).

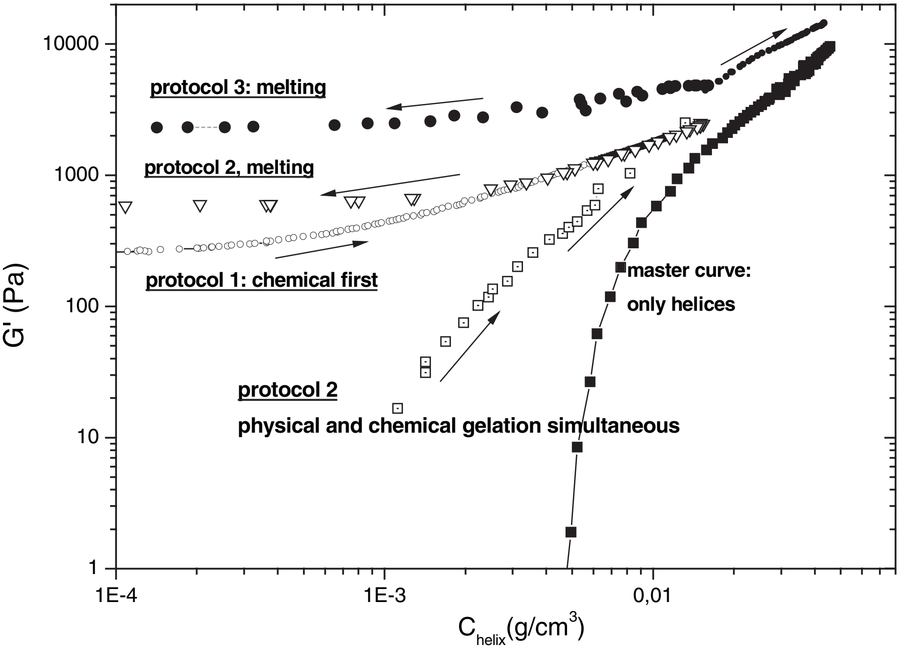

Figure 5.
The hydrogels. The 3 protocols were investigated for the same amount of BVSM ($c_{BVSM} = 0.225\%$).

the cooling step is however above the simple addition of the storage moduli of the physical and chemical gels.

The melting observed, when the gel was made by Protocol 2 shows, at the end of the heating ramp, a higher modulus than when the crosslinking was performed randomly (570 Pa). The melting at the end of Protocol 3, gave even a higher modulus (2260 Pa). In all cases investigated, the helices have disappeared at the end of the heating ramp. In conclusion, we see that crosslinks made in presence of triple helices are probably unevenly distributed and this provides a more efficient contribution to elasticity. We also suppose that if hydrogels have a higher modulus than physical gels, this is because the crosslinks provide more rigid junctions between the helices, in particular when the crosslinking occurs in presence of helices. In Figure 5, the contribution to elasticity is larger, for the same amount of helices, when the crosslinks were made after the physical gel.

More theoretical work is needed to interpret the relation between rheology and structure in hydrogels.

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